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RESEARCHES ON THE ALLEGED INFLUENCE OF SYMPATHETIC INNERVATION ON WARMTH PRODUCTION IN SKELETAL MUSCLES

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The question of the so-called autonomic innervation of the muscles entered a new and more decisive phase when Boeke (1) and his pupil Agduhr conclusively demonstrated by means of accurate experiments of an anatomical character what they called the sympathetic innervation of the striated voluntary muscles. The physiological work which had been done before these findings had been very suggestive, but had been lacking in secure foundation, but now many questions demanded solution in view of the possibility that the sympathetic nerves had an influence on various functions of the muscles.

A review of the literature of the past few years bearing on this subject shows, however, that rather a majority of exact experimental investigations indicate that such nerves certainly have no definite influence on the motor functions of the muscles—not even on those functions grouped under the term "tonus." Some recent experiments carried out in the Berne Physiological Institute on this subject also led to the conviction that there is no permanent effect on the motor functions of the muscles if the whole sympathetic innervation to those muscles has been taken away. Observations made during these experiments support this view as well. There remains as the only possibility, therefore, of vindicating the belief in the tonic influence of the sympathetic nerves on the voluntary muscles, the theory that the loss of it is easily and perfectly compensated by the far stronger tonic influence of the efferent motor nerves proper.

By far the most interesting problem, however, which presented itself for solution as soon as one accepted the work of Boeke (1) was the much-

debated trophic or metabolic influence of the nervous system on the muscles. It had formerly been thought that even without contraction of the muscle nervous impulses reaching the muscle could augment its metabolism. According to this view one believed that there might be the formation of warmth in the muscles under the influence of the nervous system which was separable from warmth production due to motility or contraction. Experimental evidence failed to support this view, but after the presence of sympathetic nerves to the muscles was satisfactorily proven, there was a renewal of effort to show that impulses reaching the muscles by way of the sympathetic pathway influenced the metabolic processes in the muscles. Besides the researches of Leonhard Hill (2) and his collaborators (3) perhaps the most important contribution is that of Freund and Jansen (4). The latter come to the conclusion that the sympathetic nerves play a part in the chemical regulation of warmth in the muscles, and prove it in the following way. They investigate the oxygen consumption in a muscle of the cat isolated according to the method of Barcroft and Verzar. In an animal which regulates its warmth the oxygen consumption of the muscle rises even after cutting the motor nerves when the animal is kept cool, and sinks when it is overheated. Moreover, this regulation is maintained after extirpation of the thyroid gland, but they found it disappeared after what is now commonly known as the Le Riche operation (a localized periarterial denervation) had been done. It is easily seen that the authors come to this conclusion on the basis of the assumption that the sympathetic nerves going to the muscles do not find their way by the peripheral nerves, but by way of the large arteries. This assumption appears something more than doubtful. Certainly there are regions of the body, notably the salivary gland, liver or kidney, where autonomic nerves reach the tissue which they innervate at least partially by way of the blood vessels. For it is well known that if one wishes to denervate totally these glands it is necessary to treat the blood vessels entering them with a substance which will perfectly destroy the nerves accompanying the blood vessels which supply the organs. But in the case of the muscles, especially those of the extremities, the situation is quite a different one. Quite recently, at a time when the experiments reported here were nearly finished, the first authority on the anatomy and physiology of the sympathetic system, J. N. Langley (5), has once more investigated the question of the path taken by the sympathetic nerves peripherally, coming to the conclusion that if periarterial section in man relieves peripheral vasoconstriction, it in all probability does not do so by severing nerve fibers running with the arteries toward the periphery. According to Langley, there are no facts known which justify any alteration of the plan of sympathetic innervation outlined so lucidly by him many years ago.

It appears, therefore, that the theoretical basis of Freund's paper, of the papers of some other authors, and also the original assumption that the results obtained by Le Riche and his followers are due to an interruption of sympathetic nerves running with the arteries, can not be maintained. The facts reported, however, might be as is often the case, independent of any theory, correct.

At the suggestion of Prof. Leon Asher the following investigation was undertaken to still further elucidate the question of the sympathetic influence on the muscles, a method being employed different from the one used by Freund and Jansen. In order to deprive a part of the body completely of its sympathetic innervation and then to compare it with the normal side in the same animal, the whole sympathetic chain on one side of the body in the lumbar and pelvic regions was removed, thus taking away the entire sympathetic innervation of the lower extremity on that side. To test the effect of this operation on the function of the muscles on the denervated side compared with that of the normal side, puncture of the thermal center in the corpus striatum was chosen. The object of this experiment was to cause a rise of temperature which would affect both normal and operated sides, and thereby enable us to observe any differences between the two. If the impulses coming down the sympathetic nerves caused the muscles to partake in the heat regulation of the body, or caused at least an augmentation of the processes appertaining to such regulation in muscles, then on the normal side the course and size of temperature variation after puncture ought to be different from that on the denervated side.

With the use of this method ("warmth-puncture"), employed by so many investigators, one touches a problem very much debated and related to that which is the subject of this paper, namely, the part played by the voluntary muscles in warmth regulation. Two opinions are upheld, the one that the muscles only partake in warmth regulation insofar as by motor impulses they are caused to contract or alter their state of tension, the other that independently of any mechanical change in the muscle by nervous impulses the processes leading to warmth regulation could be altered. Only those investigations which employ "heat-puncture" as a method of testing which of the two opinions might be correct will be considered here. Ito (6), working under Kronecker in the Berne Physiological Institute, concluded from the fact that the rise of temperature measured in a duodenal fistula was the largest, that processes in the glands played the greatest part in the effects of warmth-puncture. In a more direct way he was followed by Linelnikow (7). The latter used different methods to exclude the influence of the thermal center on the muscles. He cut the spinal cord between the fourth and fifth lumbar vertebrae and the brachial plexus on both sides. He also cut the spinal

cord as high as between the seventh and eighth thoracic vertebrae. In both cases he obtained the same increase of temperature after warmth puncture as in the normal animal, but when he cut the spinal cord between the second and third thoracic vertebrae, the warmth puncture was without effect. From these results he concluded that the origin of increased warmth after puncture of the thermal center lay not at all in the muscles but in the large glands of the body. These results of the Kronecker school were in accord with many attained by using metabolism experiments. But this is another field. Inasmuch as none of the latter methods were used in this research no attempt is made to consider the very extended literature of this kind. Nor does it seem necessary for the purposes of this report, as mention has already been made of Freund's work, which appears to be the only series of experiments in which both the problem of heat regulation in the muscles and that of sympathetic innervation of the muscles has been combined, and that in a way unknown before the Le Riche operation connected the two.

METHODS OF INVESTIGATION: *a*. Animals used were rabbits, all males.

b. Extirpation of lumbar and pelvic sympathetic chain.

The operation described in the following paragraph is in nearly all essential details the same as that of E. Schmid (8) except for certain variations in technic.

Anesthetic, 2 cc. of 2 per cent morphine sulphate subcutaneously given, produced a deep narcosis lasting from one to two hours.

With the animal securely tied on its back, the skin and peritoneal incisions were made exactly in the linea alba, and carried from ensiform to symphysis, care being taken not to injure the bladder. As complete evisceration as far as the intestines are concerned is absolutely necessary to a thorough extirpation of the lumbar sympathetic chain, great care must be taken that they are not handled excessively or allowed to cool. They were therefore first wrapped in a sheet of fine "rubber-tissue," covered with absorbent cotton which was kept warm by means of hot saline solution, and held on top of the anterior chest wall. In this way all injury of even the most minor degree to the peritoneal surfaces was avoided, and active, rigorous peristalsis always seen when the bowels were returned to the abdomen. If gentle traction be now exerted on the sigmoid loop of gut, the film-like peritoneum at the root of the mesentery is put on a stretch and a rent may be readily made in it. One now sees the sympathetic chain lying behind and to the side of the aorta and vena cava, running between the lumbar arteries and veins. With a fine blunt-pointed instrument the ganglia are now freed from between these vessels and the rami communicantes carefully cut. It is possible to free thus the entire cord from the fourth lumbar to the third sacral, but the greatest care must be taken not to injure or even touch the sympathetic chain on

the other side, which lies exceedingly close. No difficulty should be experienced thus far though the last lumbar ganglion lies in such close proximity to the sacralis media artery with the ileo-lumbar vessels over it that the greatest care must be taken in loosening it. (The slightest hemorrhage at any time will so obscure the field as to forbid continuance of the operation, and will in addition endanger the life of the animal because of the difficulty of controlling it here.) The removal of the sacral ganglia will be made materially easier if the lumbar chain has been preserved as a guide. The first sacral ganglion lies in the midline on the brim of the promontory of the sacrum and close to the sacralis media artery. The second ganglion lies in the same location, but slightly lower. At this point one must mobilize both bladder and rectum with their vessels and nerves in order to follow the chain down the very narrow pelvis. The third sacral ganglion lies behind the venous plexus which

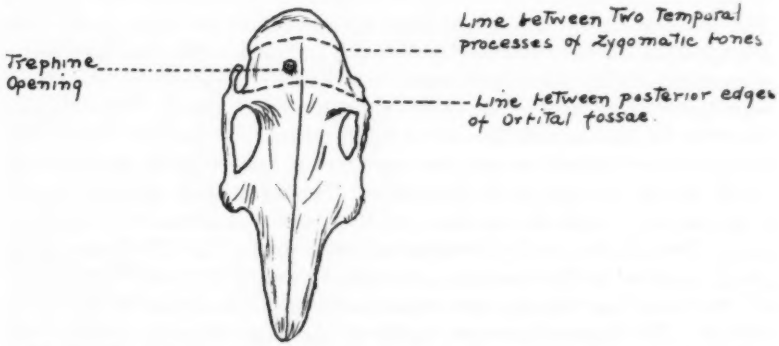

Fig. 1. (Reproduced from Aisenstadt; Arch. f. Anat. u. Physiol., 1909, 475.) Schematic diagram of skull of rabbit to show localization of trephine opening.

fills the pelvis and is very closely attached to the medial sacral artery as well as to the spinal nerves which come from the cord at this point. This is the most trying and difficult ganglion to remove in the whole lumbar and pelvic chain. Replacement of the mobilized parts, closure of the peritoneal rent with a few sutures of very fine black silk, returning of the intestines to the abdomen and closure of the abdominal wound in layers with interrupted sutures of black silk completes the operation. It should be absolutely bloodless from the first skin cut to the last suture, nor does it seem possible to be sure of a complete extirpation in the face of even the slightest hemorrhage. Strict asepsis is of course imperative.

c. Puncture of the thermal center (*"warmth-puncture"*). The method here employed differs only from that of Ito (6), of Nyffenegger (9) and of Aisenstadt (10) in that the animals were tied securely on a board,

thus permitting of more rigid asepsis, and leaving both hands free for the novocanization of the skin and the sewing of the wound. The entire operation is completed in about ten minutes, from sterilization of the skin to collodion dressing. If two imaginary lines are drawn, *1*, connecting the posterior edges of the orbital fossae and *2* between the temporal processes of the zygomatic bones, the point selected for the trephine opening lies posterior to the anterior of these lines about one-third of the distance between the two, and 3 mm. to the side of the mid-line. One per cent novocain is used in the skin, and a short incision 1 cm. long made through skin and periosteum. The trephine is then done, a small slit made in the dura in an avascular area and the puncture needle passed straight downward in a perpendicular direction to the base of the skull. Periosteum and skin are then closed and a collodion dressing lightly applied.

d. Measurement of temperature. Three very carefully graded and very sensitive thermometers were used[1] to measure the temperature in rectum, left and right legs. The tips of these thermometers are quite small, thus permitting their insertion readily beneath the skin. The capillary had a diameter of 0.1 mm. and each degree was divided into tenths. They have repeatedly been proven sensitive enough in the Berne Physiological Institute to demonstrate the rise of temperature in a muscle of the rabbit during a short tetanic contraction, and are the same type of thermometer as M. Smith (11) and S. M. Lukjanow (12) used in their work on muscle temperature. A point low down on the thigh was selected, a small incision through skin and subcutaneous tissue made, and the thermometer gently inserted in this opening and pushed upward for a few centimeters so that it lay beneath skin and subcutaneous tissue and just on top of the muscle. All observations were made at the same time, in rectum, left and right legs, and to eliminate the possibility of error, the thermometers were tested against each other before each experiment, and also frequently shifted from one side to the other.

Post-operative findings. Of primary interest as regards immediate post-operative symptoms, following extirpation of the sympathetic chain on one side, is the state of tonus existing in the two legs. The whole question of tonus of skeletal muscles is excellently reviewed together with the entire literature on this subject by Spiegel (13) in his textbook published quite recently. From the very beginning, with any of the usual methods of gross observation, no difference whatever was observed between the tonus of the normal side, and the side without sympathetic innervation. Schmidt (8) had seen, for a few days after operation, a slight loss of tonus, but he distinctly states that after a few days invariably this difference disappeared. It seems possible to explain this variation on the basis of a difference in technic during the operation. As has been

[1] Made by Goetze, Leipzig.

stated, numerous motor nerves lie very close to the field of operation, which one must be extremely careful not to injure or even to disturb. Moreover, the findings here reported as to the lack of influence of the sympathetic nerves on what is commonly called tonus of skeletal muscles is in harmony with the results of various authors who could not corroborate the assertions that these nerves had a positive influence on tonus (13). They also agree with the findings of Fujimori (14) (working at the same time in the Berne Physiological Institute) who was able to demonstrate the lack of any sympathetic influence on the tonic reflexes of the eye muscles of the rabbit. It seems unnecessary to remark that the extirpation of the lumbar and pelvic sympathetic chain was complete. During the whole time of observation, the side without sympathetic, if the animals had not been subjected to some special experiment, was warmer to palpation and to exact measurement than the normal side, due to the persistent dilatation of the blood vessels in consequence of the loss of vasoconstrictor impulses by way of the sympathetic nerves.

In most cases the puncture of the thermal center led to the well-known and often-described consequences. Only in the first few experiments was the effect of the puncture much less marked than had been expected, and later work demonstrated quite satisfactorily the reason for this. As has been described, two very fragile and sensitive thermometers were placed between skin and muscles. It seemed worth while to attempt to make observations of a continuous character with the animal tied on a board, in order to safeguard the instruments. But in spite of the fact that the rabbits were very carefully wrapped in cotton and kept as warm as possible without affecting the temperature record, nevertheless the temperature, especially of the immobilized extremities, fell, so that in the best of these first experiments there was obtained only a well-marked initial rise of temperature following puncture which was thereafter neutralized by the cooling of the extremities. But though in quantitative and sustained rise of temperature the results were not satisfactory with these tied-up animals, as far as the principal question was concerned—the comparison between the course of temperature rise and fall in the two legs—the evidence was very conclusive.

The results demonstrated in these protocols and curves certainly do not show that the sympathetic innervation has any influence on warmth regulation in the muscles. If Freund's (4) conclusion that under the influence of such nervous impulses there was a greater oxygen consumption and therefore a greater heat production was correct, there should have been a larger rise and perhaps also a faster one on the side with the sympathetic nerves intact. The possibility that the method used here would not show a sufficiently fine temperature difference can easily be excluded. In the first place, it did show a permanent temperature dif-

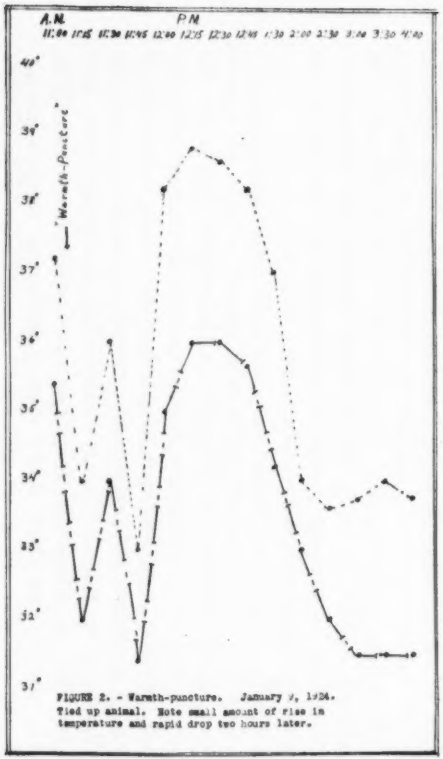

FIGURE 2. - Warmth-puncture. January 9, 1924. Tied up animal. Note small amount of rise in temperature and rapid drop two hours later.

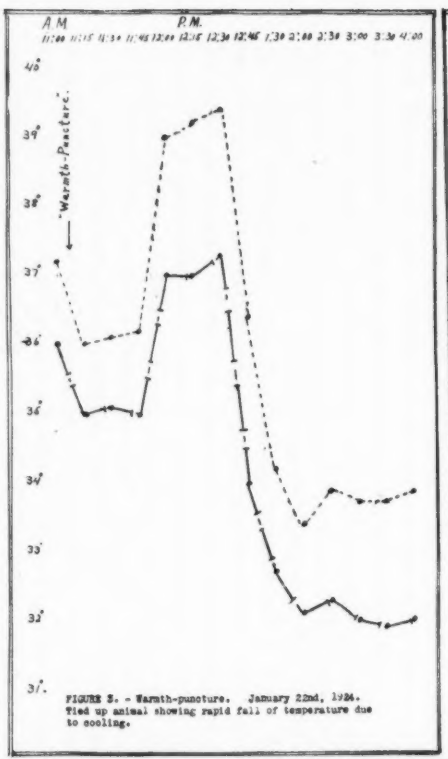

FIGURE 3. - Warmth-puncture. January 22nd, 1924. Tied up animal showing rapid fall of temperature due to cooling.

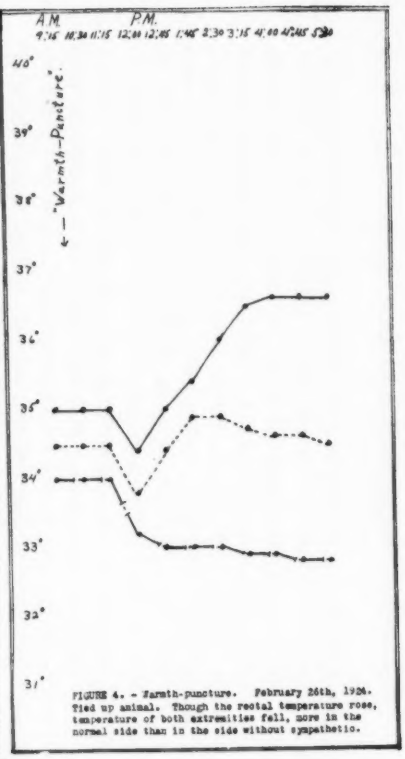

FIGURE 4. - Warmth-puncture. February 26th, 1924. Tied up animal. Though the rectal temperature rose, temperature of both extremities fell, more in the normal side than in the side without sympathetic.

Rectal Temperature ————
Temperature of Left Leg (without sympathetic) - - - - - -
Temperature of Right Leg (normal) ——— — ———

Table 1

ference; and in the second place, as has been mentioned, the comparatively small rise of temperature during the few seconds of a tetanic contraction is readily shown by this method (11).

In view of the slightly disappointing results following puncture in degree of temperature rise and persistence of the same, keeping the animals

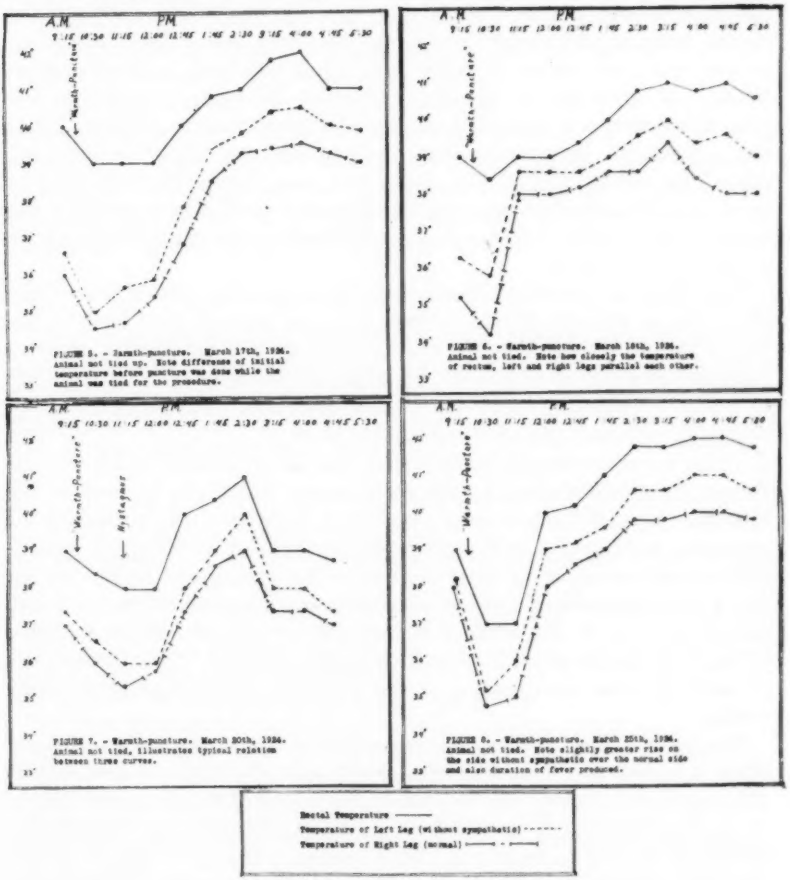

FIGURE 5. - Warmth-puncture. March 17th, 1936. Animal not tied up. Note difference of initial temperature before puncture was done while the animal was tied for the procedure.

FIGURE 6. - Warmth-puncture. March 18th, 1936. Animal not tied. Note how closely the temperature of rectum, left and right legs parallel each other.

FIGURE 7. - Warmth-puncture. March 20th, 1936. Animal not tied, illustrates typical relation between three curves.

FIGURE 8. - Warmth-puncture. March 25th, 1936. Animal not tied. Note slightly greater rise on the side without sympathetic over the normal side and also duration of fever produced.

Table 2

tied up was abandoned and observations made less frequently (every 45 minutes), allowing the rabbit to move about between each observation. From the moment this regime was instituted the results were in every way satisfactory. Heat puncture led to a high and long-lasting rise of temperature measured in the rectum and in the legs.

We now see clearly demonstrated in these charts that in consequence of puncture of the thermal center a very rapid and sustained rise in temperature of considerable degree, which is present in all three places measured. The relationship in these three locations is almost identical, the rectal temperature being the highest, that of the side without sympathetic next and the normal side lowest. A comparison between the curves from the two legs reveals not one case where the rise on the normal side was higher or more rapid than on that without sympathetic. If anything can be said as to the difference it is that on the denervated side now and then the temperature showed a slightly higher rise. When the temperature was observed the day after the puncture, almost invariably the effects of the puncture were still noticeable in more or less marked degree, and the described proportions remained the same. This is in favor of denying any influence of the sympathetic nerves on warmth regulation.

Regarding the possible objection to the conclusions drawn from these observations that the failure to show a larger production of heat on the side with its sympathetic innervation because this production is overbalanced by a failure to give off the heat, one can only say that there are so many hypothetical elements in this objection without any basis of observed facts that one need only allude to it.

These experiments also exclude the possibility of there being any influence from hypothetical sympathetic nerves which run by way of the arteries. Even if there were any such they have been interrupted by the operation performed. It is obvious that extirpation of the entire sympathetic chain immediately after its leaving the spinal cord in the lumbar and pelvic regions would cut as effectively any sympathetic nerves which passed by way of the arteries toward the peripheral muscles as those sympathetic paths which have so long been recognized. No attempt here is made to open the question of the so-called para-sympathetic innervation.

During one phase of these experiments an attempt was made to achieve what is known as chemical fever, for the sake of additional and slightly different observations. To be reliable as a means of comparison a method of causing chemical fever which would be capable of exact quantitative measurement had to be found. This precluded the use of any substance not pure chemically and not capable of such estimation. Saline solutions of various concentrations were therefore used, being injected into the ear vein of the rabbit. Not being satisfied with the outcome of these efforts, they were abandoned. Mention may be made, however, of some constant observations which seem to merit later investigation. Invariably when the period of fall of temperature came, which was always seen in the tied-up animals, the drop on the normal side was always

considerably larger than on the denervated side. The simplest explanation of this finding seems to be that in consequence of the injection of more or less concentrated saline solutions there develops a state of increased tonus of the vaso-constrictor centers leading to stronger vaso-constriction on the side receiving its normal sympathetic impulses.

SUMMARY. A method was devised whereby the whole lumbar and pelvic sympathetic chain on one side in the rabbit was extirpated without damage to adjacent motor innervation.

This extirpation had no observable influence on either the motility or tonus of the muscles on the side of operation; and puncture of the thermal center in the corpus striatum led to a rise of temperature observed in the rectum and in both extremities, without showing a larger or more rapid rise on the normal side—if anything a somewhat greater rise in some cases on the side without sympathetic innervation.

CONCLUSIONS

These facts indicate that sympathetic innervation has no influence on heat production in skeletal muscles, and are also opposed to the belief that there are such nerves having an influence on warmth regulation which reach the periphery by way of the arteries. This latter conclusion is in accord with the opinion of E. N. Langley.

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QUANTITATIVE MEASUREMENTS OF THE EXCITABILITY OF THE CENTRAL NERVOUS SYSTEM AFTER THYROIDECTOMY AND THYMECTOMY

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As Asher (1) has expressed it in his Harvey Lecture, the central nervous system is for the thyroid gland a peripheral structure. Undoubtedly the thyroid gland has an influence on the central nervous system. Much experimental and clinical evidence has been accumulated which tends to show a decrease in nervous activity in organisms having a deficiency of the thyroid gland and there is some evidence of increased activity after feeding the active principle of this gland and in Grave's disease. Quantitative evidence is however rather meager. In order to obtain such exact measurements it is necessary, in accord with the usual physiological routine, to study intensively the variation in excitability of some part of the nervous system in its relation to the gland.

In 1920, Ruchti (2), working in the Berne Physiological Institution, made the following casual observations during his work on the influence of the thyroid gland on respiratory metabolism. He found that his animals seemed to react much less after thyroidectomy when they were warmed, or in other words, that the frequency of respiration was augmented much less to gross observation in an animal without thyroid than in the normal animal when the temperature of the surrounding air was increased. He used rabbits, keeping them in a glass container connected with his metabolic apparatus and heating the glass chamber to increase atmospheric temperature. Still more significant was his further incidental observation that after combined removal of thyroid and thymus the decrease of excitability of the respiratory center against warming was far more pronounced and appeared to be permanent.

This work, undertaken at the suggestion of Professor Asher, is an effort to measure exactly by means of instruments of precision, the actual amount of variation in respiratory rate in the normal animal, the rabbit without thyroid, and after total removal of both thyroid and thymus, when the temperature of the atmosphere in which the animal is placed is raised or lowered.

DESCRIPTION OF APPARATUS. *a. Warming chamber.* An asbestos-covered box,[1] having variable ventilation openings, an opening through which a thermometer could be inserted, and one through which the tube to the recording apparatus passed was utilized, large enough to place the animal tied upon a board inside. This chamber was heated by electricity and any degree of desired temperature could be maintained indefinitely by means of an automatic cut-out which could be adjusted at will. We were thus able to keep the rabbit in a semi-closed receptacle from which all light and movement were excluded and into which only a minimum of sound penetrated.

b. Recording apparatus. Two small rubber tambours, connected to a rubber tube of small caliber were lightly fastened to the thorax of the rabbit, tied upon a board inside the warming chamber. Care was taken not to restrict respiratory movement by too tight application about the chest. The tube was then led through a tiny opening in the side of the box and connected to the usual tambour recording mechanism used in conjunction with the kymograph. Thus the slightest respiratory movement was transmitted in graphic record to the surface of the smoked drum.

A long and careful study was then first made of the reaction of the normal rabbit to increase and to decrease of temperature. Some facts worthy of mention in that they serve to demonstrate the extreme sensitivity of the respiratory center came to light during this part of the work. It was found that excellent records could be made by this method, but that in order to obtain anything approaching uniformity in reaction even in the same animal with experiments only a few hours apart the most scrupulous care had to be taken that conditions were as nearly similar as possible. For example, absolute silence is obligatory; and such details as a full bladder or stomach may produce quite a different picture than that present when they are empty. If the rabbit is restless and excited all attempts to obtain satisfactory records are useless. Quite by accident it was also discovered that if the animal were kept in a warm or a cold room the night before an experiment, surprisingly different results were seen the following morning. Differences in age, sex, and so on, may produce a uniformly more rapid or slow record under identical conditions. One also gets one picture if observations are started at a high temperature, the latter being slowly lowered, and another if a beginning is made at room temperature and then gradually raised. After a long series of experiments on normal animals it was found that maintenance of any given temperature for four minutes would give a typical reaction, and that all records for temperatures above 35°C. were so variable that the figures were not reliable. In all our

[1] Loaned through the kindness of Dr. R. Isenschmid of Berne.

experiments, therefore, we have observed reactions to temperatures between 20° and 35°C., each temperature being maintained for four minutes.

If now the above-mentioned precautions are observed, it is possible to obtain eminently satisfactory curves, which demonstrate a rapidly rising respiratory rate in the normal animal when it is subjected to differences in temperature (cf. table 1). The greatest variations occurred in these animals between the temperatures 22° and 23°; and after reaching a warmth of 30° there was but little change. Viewed as a whole, one sees an enormous rise in rate, from 7 to 47 per ten seconds.

Total extirpation of the thyroid was then done and exactly similar observations made.

DESCRIPTION OF OPERATION. *Complete thyroidectomy.* Anesthetic 2 cc. of 2 per cent morphine sulphate, subcutaneously given.

TABLE 1

Respiratory rate of normal rabbit in varying degrees of external heat

(Rate per ten seconds)

	TEMPERATURE OF AIR															
	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°	35°
Rate of respiration per 10 seconds	4	8	9	15	22	22	25	28	34	38	44	45	45	45	48	46
	6	7	9	16	21	23	24	27	35	37	43	46	46	48	49	49
	4	7	8	16	20	23	25	28	32	36	42	47	46	48	48	48
	6	8	8	10	20	22	25	29	35	39	44	46	46	48	48	46
	7	8	9	16	22	23	25	27	33	36	42	45	45	47	47	48
Average	7	8	9	15	21	23	25	28	34	37	43	47	47	47	48	47

Thyroidectomy in the rabbit is a very simple procedure, requiring at most only ten minutes, and being quite bloodless. A small incision (2 cm. long) is made in the mid-line of the neck, just over the cricoid cartilage and carried through the subcutaneous tissues and pretracheal muscles, exposing the trachea. The thin, film-like isthmus of the gland is immediately seen lying over the trachea, with the left and right lobes of the thyroid on each side. Ties of very fine black silk should be placed about the blood vessels at each pole and scrupulous care observed to see and avoid the recurrent laryngeal nerves which lie very close. Rabbits do not support injury to even one of these nerves well. If there be a slight oozing from the anterior surface of the trachea from very fine anastomatic branches here, no alarm need be felt, for replacement of the pretracheal muscles will absolutely control this. It is futile to attempt to clamp or tie in this region. After being sure that all the gland has been removed, a few sutures of fine black silk in muscles and skin, and a collodion dressing complete the operation.

Four days were allowed for recovery from the effects of the morphia and operation, and then the animals without thyroid were observed in the same way as before.

One now sees (cf. table 2) a marked lowering of respiratory rate for all temperatures above 22°, and a range of variation of from 6 to 34 per ten seconds as opposed to 7 to 47 for the normal animals (compare with table 1). It may also be added that these records were much easier to obtain than before thyroidectomy inasmuch as the animal reacted less acutely to extraneous factors.

To thyroidectomy was now added complete extirpation of the thymus, and after allowing for recovery from the operation, the experiments were once again repeated.

Complete thymectomy. Anesthetic 2 cc. of 2 per cent morphine sulphate given subcutaneously.

TABLE 2

Respiratory rate of rabbit after thyroidectomy in varying degrees of external heat

(Rate per ten seconds)

	TEMPERATURE OF AIR															
	20°	21°	22°	22°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°	35°
Rate of respiration per 10 seconds	6	7	8	10	14	17	20	20	21	22	28	29	32	30	32	32
	6	9	10	12	15	18	23	25	28	33	33	35	36	36	36	37
	6	9	10	12	14	14	14	15	18	20	30	33	32	33	33	33
	7	9	10	11	13	12	17	18	19	20	24	27	28	29	30	36
	6	8	10	11	14	16	16	17	19	22	22	26	28	29	30	30
Average	6	8	10	11	14	15	18	19	21	23	27	30	31	31	32	34

Incision about 4 cm. long from sternum upwards. Carried through subcutaneous tissues and muscles in the mid-line of the neck. Some authors recommend removing a part of the pretracheal muscles in order to obtain better exposure, but we did not find it necessary. A blunt hook caught under the upper end of the sternum and raised sharply provides quite sufficient exposure. The upper pole of the thymus will be found burrowing beneath the sternum and in close apposition to the large arteries of this region. If this pole is seized with broad smooth forceps and gentle traction applied the entire gland may be delivered easily from its normal location. There is always a surprising amount of gland, but very few attachments of importance. One large vessel will be found usually on the posterior surface of the thymus. This should be ligated. Care must be taken as well to avoid injury to the pleura (which is always drawn up into the wound by the traction) but a small hole in the membrane is not necessarily fatal. It should be closed, however, by a purse-string of fine

black silk. Closure of the muscle layers and skin is done as in the thyroidectomy and collodion dressing is applied.

After the double operation one sees a still greater diminution in respiratory rate. Increase of rate comes much slower and only reaches about half the rapidity of the normal animal (cf. table 3 and compare with tables 1 and 2) and approximately two-thirds as much as in the rabbit with thyroid removed.

TABLE 3

Respiratory rate of rabbit after thyroidectomy and thymectomy, under varying degrees of external heat

(Rate per ten seconds)

	TEMPERATURE OF AIR															
	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°	35°
Rate of respiration per 10 seconds	8	7	8	7	8	9	8	8	8	9	11	11	12	20	21	22
	5	5	5	5	5	5	6	6	7	6	7	8	9	12	13	14
	5	5	5	6	6	6	7	6	8	8	9	10	11	11	11	13
	6	6	6	6	6	7	8	9	10	11	11	22	22	27	28	32
	5	5	6	7	9	7	9	10	14	18	22	22	23	23	27	32
Average	6	6	6	6	6	7	7	8	9	10	12	14	15	18	20	22

TABLE 4

Respiratory rates of rabbit after giving $\frac{1}{20}$ mgm. of morphia per kilo in normal animal, in rabbit after thyroidectomy, and after thyroidectomy and thymectomy

(Rate per ten seconds)

	TEMPERATURE OF AIR															
	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°	35°
Normal	7	8	8	8	8	9	9	9	10	10	10	11	12	13	13	14
After thyroidectomy	6	6	7	7	7	7	8	8	8	9	9	9	9	10	10	10
After thyroidectomy and thymectomy	6	6	6	6	6	6	6	7	7	7	7	7	8	8	8	8

All figures are averages.

The results obtained and their striking resemblance to the well-known effects of morphia on the respiratory center led to the administration of a minute dose ($\frac{1}{20}$ mgm. of the drug per kilo of body weight intravenously) of morphine sulphate in one complete series of these experiments. Here again the records seem quite striking (cf. table 4).

It was found that an amount of morphia insufficient to reduce the rate of respiration at room temperature, would however affect the rate at higher temperatures in a striking degree, and that this reduction of excitability

of the respiratory center was again more marked in an animal without thyroid as compared to the normal, and that when both thyroid and thymus were removed there was practically no increase in rate with rise in temperature.

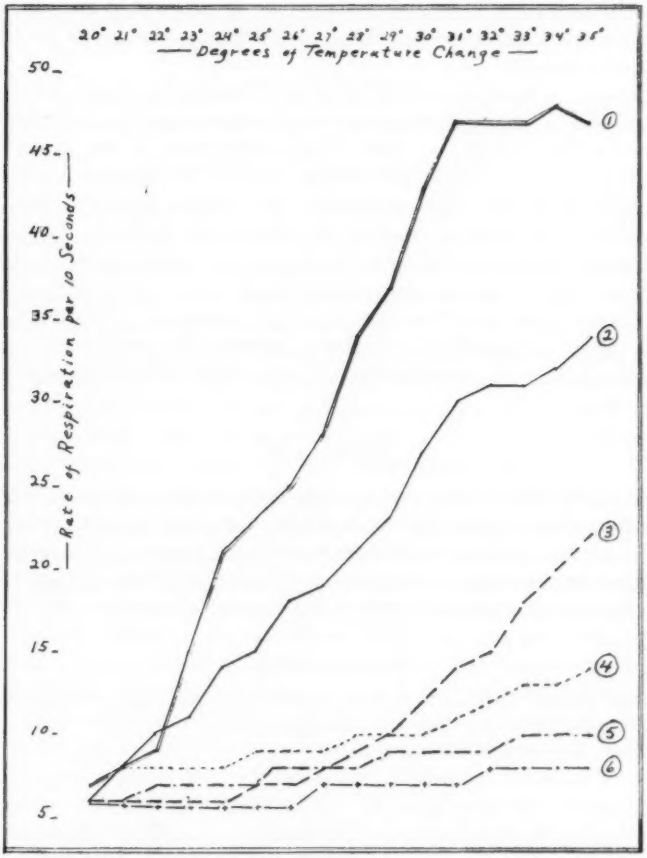

Fig. 1. Graphic representation of tables 1 to 4. *1*, Curve of respiratory rate of normal animal; *2*, of rabbit without thyroid; *3*, without thyroid and thymus; *4*, normal rabbit with $\frac{1}{2}\frac{1}{0}$ mgm. morphia; *5*, animal without thyroid with morphia; *6*, curve of respiratory rate of rabbit without either thyroid or thymus and with morphia.

One sees demonstrated, therefore, by this exact quantitative method of observing the degree of heat polypnea comparatively in the normal animal, the rabbit without thyroid, and again without either thyroid or thymus, a

very apparent influence of these glands on the excitability of the respiratory center. Certainly, also, in the animals used (rabbits), there is an augmentary interaction in their influence on this center. These facts also support in some measure the belief that there may well be a similar dependency of functional excitability of the whole or at least of the vegetative part of the central nervous system on the internal secretions of the thyroid and thymus glands.

SUMMARY. A method is here presented whereby the respiratory movements of the rabbit under various temperatures may be accurately and graphically demonstrated. After total extirpation of the thyroid the susceptibility of the respiratory center to rise of temperature becomes markedly diminished. This decrease is still further increased when total extirpation of the thymus is added to that of the thyroid. Intravenous administration of a small dose of morphia, while reducing the increase in respiratory rate to rising temperature in all three cases, still does not obliterate the essential differences observed in animals who are drug-free. Further, there is a striking resemblance between the reaction of a normal animal to whom such a dose has been given and an animal without thyroid and thymus.

CONCLUSIONS

Total extirpation of the thyroid and thymus diminish the excitability of the central nervous system toward environmental stimuli. This diminution may be quantitatively measured by the method employed here, which also demonstrates an augmentary interaction of thyroid and thymus on the functional activity of the central nervous system.

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PARADOXICAL CARDIAC INHIBITION FOLLOWING LESIONS OF THE PROPRIOCEPTIVE PATHS

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In a previous contribution (1) the author showed that paradoxical cardiac inhibition (hypersensitivity of the intra-cardiac vagal mechanisms to pilocarpine, etc.) might be induced by lesion of the efferent vagal paths. The present contribution shows that the phenomenon may be induced by lesions of the proprioceptive system.

Method. This consisted of dividing the tendons or otherwise injuring, on one or both sides of the body, certain structures known or supposed to be well supplied with proprioceptive mechanisms, e.g., the external ocular muscles, antigravity muscles, otic labyrinth, etc. For the reasons advanced in the previous paper (1) one sided lesions, except in localities where marked proprioceptive condensations occur, such as in the otic labyrinth, etc., were frequently found to be incapable of inducing the phenomenon unless one vagus nerve were also divided or injured. After these lesions the sensitivity of the intra-cardiac vagal mechanisms was tested by intravenous injections of pilocarpine hydrochloride and by other agents and the effects upon the blood pressure, etc., recorded. Ether anesthesia. Cats were mainly used but studies were also made on dogs. In 8 normal cats averaging between 6 and 8 lbs. weight, pilocarpine in repeated doses ranging from gr. $\frac{1}{60}$ to gr. $\frac{1}{30}$ failed to elicit appreciable slowing of the heart beat (1). One cat 6 lbs. and one dog 15 lbs. both supposedly normal gave a fairly well-marked inhibitory response to gr. $\frac{1}{60}$ and gr. $\frac{1}{30}$ of pilocarpine respectively. In these cases the sensitivity of the intra-cardiac inhibitory mechanisms was increased by the experimental lesions to an extraordinary degree.

Experimental facts. Paradoxical inhibition was elicited after the following lesions the reaction being moderate after lesions *a* to *c* inclusive and marked in the remaining lesions: *a.* Section of *right* and *left orbicularis palpebrarum at outer canthus plus section of one vago-sympathetic* (2 animals). *b.* Division or injury of *right* and *left internal and superior recti* through a conjunctival incision plus section of *one vago-sympathetic* (2 animals). *c.* Division or injury of *right internal and superior rectus* through a conjunctival incision plus *section of left vago-sympathetic* (2 animals). *d.*

Severance on *right and left side of body* of attachment of *temporal muscle* to coronoid process of the mandible and of *masseter* to lateral surface of ramus and angle of mandible plus section of one vago-sympathetic (2 animals). *e.* Severance on both sides of body of attachment of *cervico-occipital muscles* to occiput and spinous processes of the cervical vertebrae plus section of one vago-sympathetic (2 animals). *f.* Section in *right and left* leg of *tendon of quadriceps extensor femoris* and of *tendo Achillis* plus *section of one vago-sympathetic* (2 cats, 2 dogs). *g.* Section of *left quadriceps extensor femoris tendon* and of *left tendo Achillis* plus section of *left vago-sympathetic* (1 cat and 2 dogs). *h.* Section of tendon of *right quadriceps extensor femoris right tendo Achillis* and of *left triceps cubiti* plus section of right vago-sympathetic (1 animal). *i.* Destruction of *both otic labyrinths* (1 animal). *j.* Destruction of *one otic labyrinth* (1 animal). *k.* Destruction of *one otic* layryinth plus section of *homolateral vago-sympathetic* (1 animal). *l.* Destruction of *one otic labyrinth* plus section of *contralateral vago-sympathetic* (2 cats, 1 dog). *m. Crushing of both sciatics* plus moderate injury of *right vago-sympathetic* (2 animals). *n.* Crushing of *one sciatic* plus section of *homolateral vago-sympathetic* (2 animals). *o.* Crushing of *anterior and posterior gastric walls* (6th day) plus section of *right vago-sympathetic* (1 animal). *p.* Cauterization of *posterior funiculi of cord* (7th day) between roots C VI–VII plus section of *right vago-sympathetic* (2 animals). *q.* Pneumothorax on right side (6th day) plus section of left vago-sympathetic (2 animals). *r.* Crushing of *right and left infraorbital nerves* just outside infraorbital foramen plus section of *right vago-sympathetic* (1 animal). *s.* Hemitransection of right side of spinal cord between roots Ci and ii (1 animal). *t.* Crushing of *right sciatic nerve* and *left brachial plexus* (1 animal).

The phenomenon could not be elicited or the response was doubtful or atypical after the following: *a,* Permanent *ligation* (7th day) of the colon one inch cephalad of the *ileo-cecal junction* plus section of *right vago-sympathetic* (1 animal). *b.* Crushing of one sciatic (1 animal). *c.* Section of *one vago-sympathetic* (2nd to 8th day).

After bilateral vagus section and high and low cervical spinal cord transection as well as after removal of both stellate ganglia, and after adequate injections of ergotoxine and of nicotine, the phenomenon could still be elicited by pilocarpine whereas after atropine even large doses of pilocarpine failed to materially alter the blood pressure or the rate or amplitude of the heart beats.

Discussion and summary. In figure 1 the delayed appearance and the feebleness of the paradoxical reaction as compared with the reactions in figures 2 and 3 seem to indicate that after lesions of the proprioceptive system a period of incubation or latency occurs during which the sensiti-

zation of the intracardiac vagal mechanisms develops. Evidence of this incubation period was also found after section of both vagi (1).

In a previous contribution (1) it was noted that section of one vagus in the neck did not induce, within the first few days at least, hypersensitiveness to pilocarpine and this fact was attributed to the bilateral distribution of the efferent vagal fibers within the heart. The afferent vagal fibers are,

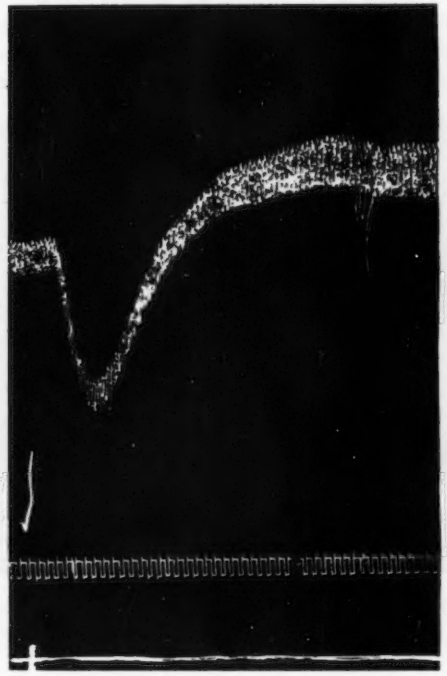

Fig. 1

Fig. 2

Fig. 1. Dog 12 lbs., 2nd day after cutting right quadriceps tendon and right vagosympathetic. Pilocarpine hydrochloride gr. $\frac{1}{100}$ at *1*. Paradoxical effects delayed. Time, seconds in all figures. Lower tracing respiratory and gastric curves from balloon in stomach.

Fig. 2. Same animal as in figure 1, 4th day. Pilocarpine hydrochloride gr. $\frac{1}{50}$ at *6*. Paradoxical cardiac inhibition.

however, to a certain extent also bilaterally distributed within the heart (11). Compare Möllgaard (2) who showed that in the lungs the afferent vagal paths have also bilateral distribution. It is possible, moreover, that the cardiac and pulmonary afferent fibers in each vagus may also have bilateral relations with the central vagal mechanisms controlling the heart.

Compare Deason and Robb (15). Such relationship would furnish a further reason for the failure of unilateral vagotomy or lesion on one side of the body, e.g., of one set of antigravity muscles, to induce marked paradoxical sensitization of the intracardiac inhibitory mechanism. In bilateral paired organs such as the eyes the unilateral distribution of the efferent dilator and constrictor pupillary paths makes it comparatively easy to induce paradoxical phenomena by cutting off completely the access of efferent impulses to the dilator or constrictor myoneural junctions of either eye. On the other hand in a single fused organ like the heart, owing not only to the bilateral distribution of the afferent and efferent vagal

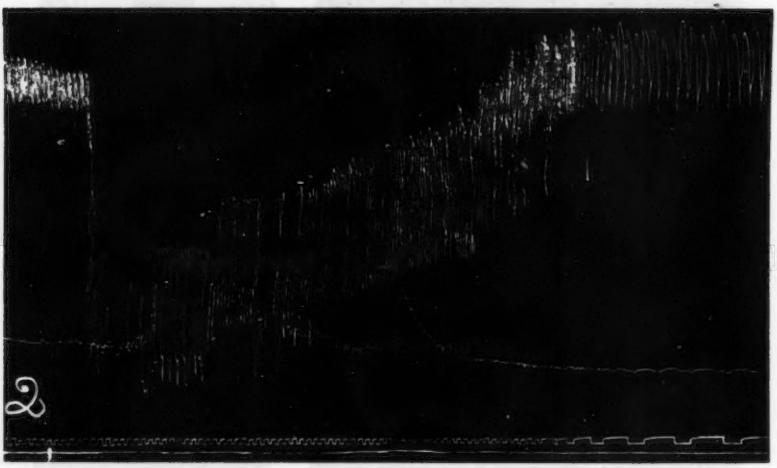

Fig. 3. Dog 12 lbs., 3rd day after cutting left quadriceps and Achilles' tendons, and 11th day after cutting right quadriceps and Achilles' tendons and right vago-sympathetic. Pilocarpine hydrochloride gr. $\frac{3}{100}$ at *2*.

(and sympathetic) nerves but also to the possible bilateral central relations of the afferent paths, abolition or reduction, by a unilateral lesion, below a certain minimum of the flow of efferent impulses to the myoneural junctions of any particular area, e.g., the pacemaker area, is more difficult of accomplishment. Section of one vagus although apparently without effect on the cardiac rhythm, etc., narrows the margin of reserve of the higher inhibitory mechanisms to such an extent that further interference with the afferent or efferent inhibitory paths becomes registrable as compensatory hypersensitization of the intracardiac mechanisms to pilocarpine, asphyxiation, etc., as well as perhaps to certain other non-neural modes of activation.

In the animals in which the otic labyrinth was destroyed the question arose: Did the labyrinthine injury involve the vagus in the vicinity of its point of exit from the cranial cavity? Upon the answer to this question depends whether the paradoxical inhibition observed after destruction of the labyrinth is to be attributed solely to the labyrinthine lesion or to the combined effects of labyrinthine and vagal injury. Injury of the vagus may, however, be safely excluded because *a*, after section of the contralateral vagus there were no disturbances of respiration such as appear after bilateral vagus section or injury; *b*, the paradoxical inhibition induced by destruction of one labyrinth became greatly enhanced some time after section of the homolateral vagus. As the phenomenon was not abolished by bilateral vagus section, by removal of both stellate ganglia, nor after high and low cervical cord transection, nor by full doses of ergotoxine and nicotine, it was concluded that the mechanisms immediately concerned in the hypersensitization process were situated within the heart presumably at the myoneural junctions.

This view was supported by the fact that after atropine the phenomenon could no longer be elicited. In the mechanism of the phenomenon as induced by lesions of the antigravity muscles, otic labyrinth, etc., the fundamental remote factor seemed to be failure of the flow of proprioceptive impulses which normally conditions the reflex outflow of cardiac inhibitory impulses over the vagi, the failure of outflow of the cardio-inhibitory impulses being itself the factor immediately responsible for the hypersensitization of the intracardiac inhibitory mechanisms. Compare Byrne (8). In the otic labyrinths are found marked condensations of critical (proprioceptive) mechanisms which control *a*, the postural adjustments mainly of the head and eyes to the neck and rest of the body (otoliths, statoliths of Verworn). Compare the stelle reflex originally described by Lyon (24) and the 4 forms of it worked out by Magnus and De Kleijn (16) and *b*, the movements by which in motion, actual or relative, balance is maintained and spatial orientation preserved through visual fixation of actually or relatively moving objects (semicircular canals). In the semicircular canals there is a preponderance of the kinetic mechanisms whereas in the otolithic apparatus the tonus mechansims are predominant. It is not surprising, therefore, to find that destruction of one labyrinth, like high hemitransection of the spinal cord, could of itself induce sensitization of the intracardiac vagal mechanisms with both vagi intact. The failure of certain lesions, e.g., section of one sciatic, to induce paradoxical cardiac inhibition whilst both vagi were intact merely indicated that the deficit of proprioceptive inflow was not, under the circumstances, sufficient to induce manifest, registrable sensitization of the intracardiac vagal mechanisms. This view receives support from the fact that after crushing of both sciatics, and after crushing the right sciatic and the left brachial plexus,

fairly well-marked paradoxical cardiac inhibition was elicited with both vagi intact. It is noteworthy that after destruction of one labyrinth the ocular nystagmus completely disappeared, and the postural deformity of the head and neck almost completely disappeared, after the lapse of some weeks. Coincident with the compensatory adjustments responsible for the animal's improvement in station and equilibration, the intracardiac vagal mechanisms seemed to lose their hypersensitiveness to pilocarpine. The failure to obtain intracardiac vagal hypersenitization after ligation of the cecum caudad of the ileo-cecal junction plus section of the right vago-

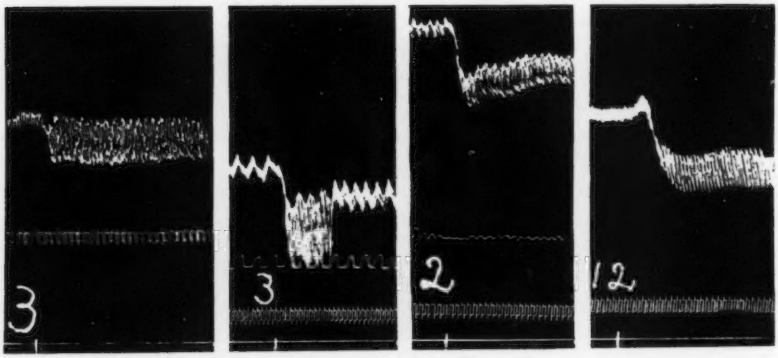

Fig. 4 Fig. 5 Fig. 6 Fig. 7

Fig. 4. Cat 6 lbs., 7th day after cutting left quadriceps and Achilles' tendons and left vago-sympathetic. Pilocarpine hydrochloride gr. $\frac{1}{50}$ at *3*. Paradoxical effects.

Fig. 5. Cat 7 lbs., 7th day after cutting right vago-sympathetic and insertions of masseter and temporal muscles on both sides of body. Pilocarpine hydrochloride gr. $\frac{3}{100}$ at *3*. Paradoxical effects.

Fig. 6. Cat 6½ lbs., 7th day after cutting occipital muscles and right vago-sympathetic. Pilocarpine hydrochloride gr. $\frac{1}{100}$ at *2*. Paradoxical effects.

Fig. 7. Cat 5 lbs., 7th day after cutting right vago-sympathetic and crushing anterior and posterior wall of stomach. Pilocarpine hydrochloride gr. $\frac{1}{200}$ at *12*. Paradoxical effects.

sympathetic must be regarded as inconclusive. In the author's opinion more work is necessary in order to reach a definite conclusion on this point.

In general the peripheral somatic nerves contain both affective and critical (proprioceptive) fibers. The proprioceptive system is, however, relatively insusceptible to ordinary electric stimulation compared with the affective system. This and the fact that in the peripheral nerves, as Ranson (12) has shown, the affective (unmyelinated) fibers are far more numerous than the proprioceptive (myelinated), are perhaps the main reasons why stimulation of a peripheral nerve such as the sciatic usually

evokes cardiac acceleration. This effect was held by many physiologists to be due exclusively to reflex inhibition of the cardio-inhibitor mechanism until Hooker (4) showed that after double vagotomy the reflex acceleration though potentially present was actually not registrable on the heart rate until this latter was kept about the normal by constant stimulation of one peripheral vagal segment. The proprioceptive system, is, however, very sensitive to its normal mode of activation, viz., alterations of tension in muscle, tendons, etc., in all of which proprioceptive (receptor) mechanisms have been abundantly demonstrated. In the present studies this fact was utilized when certain proprioceptive mechanisms were put out of commission, e.g., by lesions of the antigravity muscles, division of the tendons of which as well as of the external ocular muscles, induced hypersensitization of the intracardiac inhibitory mechanisms.

The clinically well-known functional relations between the stomach and the heart find confirmation in the animals in which vagal hypersensitization was induced by stomach lesions. Manifestly the stomach has afferent paths which control to some extent reflex cardiac inhibition. Compare the Goltz phenomenon in which cardiac inhibition is induced by tapping the stomach or intestines. Although the object in view in making the gastric lesions was to injure the vagal afferents it must be remembered that the stomach has also afferent nerves which enter the spinal cord mainly through the upper six thoracic segments and so possibly reach the cardio-inhibitory center. The results obtained after artificial pneumothorax clearly show functional relations between the pulmonic vagal fibers, more especially those activated by lung expansion, and the cardio-inhibitory center, a fact which had previously been established by Brodie and Russel (7). Besides the pulmonary vagal branches the depressor nerve, now known to be the afferent nerve of the aorta (6), exerts a powerful influence on the cardio-inhibitory center and von Brücke (3) has shown that the cardiac slowing upon central vagus stimulation is the result, in part, of stimulation of the inhibitory center and in part of inhibition of the accelerator center. On the other hand Hooker (4) has shown that under certain circumstances after double vagotomy stimulation of the central segment of the divided vagus, splanchnic, sciatic or saphenous nerve directly activates the accelerator mechanisms, whereas Bainbridge (5) found that the acceleration induced by sciatic stimulation is, in part, due to inhibition of the vagal (inhibitory) center and in part to stimulation of the accelerator center. Evidently, therefore, the movements, of the heart like those of the iris (8) are governed by two separate sets of reciprocally related neural mechanisms. One of these, the inhibitor set, is served on the efferent side by the parasympathetic (vagal) system driven on the afferent side, as the present studies show, by the proprioceptive (critical) system whilst the other, the accelerator set, is served on the efferent side by the sympathetic

system driven on the afferent side by the affective system. The affectivo-sympathetic mechanism seems to be primarily and fundamentally concerned with cardiac systole and the critico-parasympathetic system with diastole which appears to be to some extent an active process. It seems necessary to postulate for cardiac muscle at least two kinds of effector mechanism one driven mainly by sympathetic and the other mainly by parasympathetic (vagus) efferents. The former correspond to the dilator effectors of the pupil and the latter to the constrictor effectors. Confirming and supplementing the views long ago expressed by Botazzi, Fano and others and more recently by de Boer (20) the anatomical and physiological studies made by Boeke (17) and by Boeke and Dusser de Barenne (18) as well as by others seem to have shown that in striated muscle are found two anatomically separate mechanisms, viz., the anisotropic discs and the sarcoplasm which differ not only in structure, mode of contractility and metabolism but also in innervation. The anisotropic discs innervated by cerebro-spinal fibers ending in the motor end-plates mediate the kinetic elements whilst the sarcoplasm innervated by non-medullated fibers of "sympathetic nature" (Boeke and Dusser de Barenne) terminating in special end organs mediate the tonus element. But contrast Kuno (19), Cobb (21) and others whose findings, in the author's opinion, do not invalidate those of the proponents of the dualist hypothesis to which in general the present studies seem to lend support. The reflex kinetic and tonus phenomena observed in the spinal and decerebrate preparations make it appear, however, that on the efferent side the sarcoplasmatic mechanism is served mainly by the parasympathetic system driven on the afferent side mainly by the critical (proprioceptive) system whereas the anisotropic discs are innervated by the cerebro-spinal nerves driven on the afferent side mainly by the affective system. Compare Langley and Cato (22) who found that the peripheral twitchings observed in striated muscle after denervation were not abolished by atropine but were abolished by large doses of physostigmine. Compare cardiac fibrillation, etc. With the dualistic hypothesis mechanistic interpretation of the findings of these observers becomes possible. Compare further the Webers (23) who demonstrated that the inhibitory function of the vagus includes not only slowing but also weakening of the heart beat, a fact of considerable significance in the present studies since ordinary doses of pilocarpine, e.g., gr. $\frac{1}{100}$ to a 6 lb. cat causes a fall in blood pressure presumably by weakening the force of the heart beat since the rate is unaffected. Compare Byrne (1). The weakening of the force of the heart beat in such cases is to be interpreted as the result of stimulation of the parasympathetic (tonus) effector mechanisms with consequent moderate inhibition of the antagonistic sympathetic (kinetic) effector mechanisms. Where the intracardiac vagal (parasympathetic) effector mechanisms have become sensi-

tized then ordinary doses of pilocarpine, etc., have greater effects upon the parasympathetic effector mechanisms with consequent greater inhibitory effects upon the sympathetic (kinetic) effector mechanisms and how not only the force but also the rate of the heart beat becomes affected. In the pupil dilator mechanism the kinetic element overshadows the plastic tonus element whereas in the constrictor mechanism the tonus element overshadows the kinetic element. Compare the relation of the constrictor mechanism to the proprioceptive system and to the mechanism of postural tonus and cognitive protective reactions (1). The kinetic and tonus elements find representation in both the constrictor and dilator mechan-

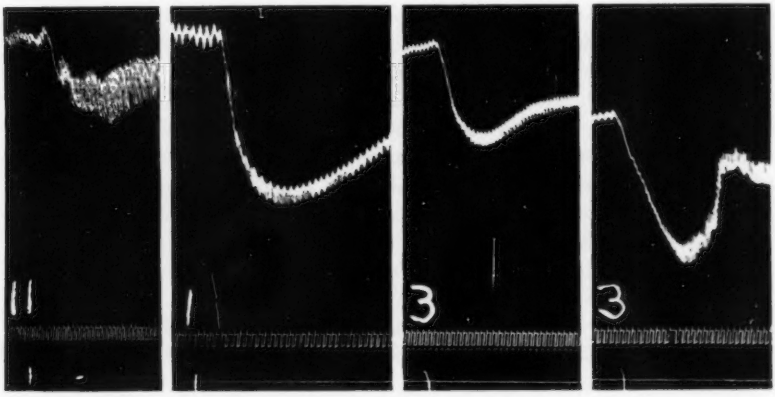

Fig. 8 Fig. 9 Fig. 10 Fig. 11

Fig. 8. Cat 7 lbs., 7th day after cutting right vago-sympathetic and crushing right and left infraorbital nerves. Pilocarpine hydrochloride gr. $\frac{1}{50}$ at *11*. Paradoxical effects.

Fig. 9. Cat 6½ lbs., 5th day after section of right vago-sympathetic and induction of right pneumothorax. Pilocarpine hydrochloride gr. $\frac{1}{100}$ at *1*. Paradoxical effects.

Fig. 10. Cat 8 lbs., 11th day after cutting left sciatic and 7th after crushing right brachial plexus. Pilocarpine hydrochloride gr. $\frac{1}{100}$ at *3*. Paradoxical effects.

Fig. 11. Cat 7½ lbs., 2nd day after hemitransecting spinal cord on right side between roots C i and ii. Pilocarpine hydrochloride gr. $\frac{1}{50}$ at *3*. Paradoxical effects.

isms in each of which, as elsewhere in striated muscle throughout the body, they seem to function side by side in a coöperation of antagonism seemingly the counterparts on the efferent side of the antagonistic afferent system, which drives them. In the heart muscle, unlike the iris, the effector mechanisms mediating the kinetic and tonus elements seem to have but a single efferent supply, viz., sympathetic for the kinetic mechanism and parasympathetic for the tonus or inhibitory mechanism. It is possible, however, that like the pupillary constrictor and dilator mechanism the tonus (inhibitory) effectors in the heart muscle fiber may receive some

sympathetic innervation and the kinetic effectors some parasympathetic innervation. Compare the reversible action of sympathetic and parasympathetic drugs (1).

The chemical activators including the hormones, etc., have perhaps been somewhat overestimated as initiators and regulators of normal function. In the normal activities of the cardiac mechanisms they play but a secondary rôle. The reactive adjustments of the fully integrated animal in response to environmental stimuli can only be adequately mediated through the central nervous system. In the light of the present studies the afferent somatic and visceral nerves seem to be primary fundamental drivers and regulators of the heart in the intact animal. Here, as in the pupillary mechanisms and elsewhere throughout the body, "the two great afferent systems the *affective* (nociceptive) and *critical* (proprioceptive) function side by side in a coöperation of antagonism each within certain limits of stimulation controlling and modifiying the other. . . . ." (8). In the fully integrated animal the affective (nociceptive) system not only mediates the objective nociceptive reactions (movements) but also the subjective reactions, viz., pain, fear, and the coarser emotions with all that these imply in the way of secondary reactions (motor, secretory and inhibitory) which act as reinforcing stimuli and so tend to intensify and prolong the original reactions (emotions, etc.). Compare the Lange-James theory of the emotions. Compare also Byrne (9). After injury the primary affective neurones are capable of elaborating and storing (10) an excess of neural energy (neuro-potential) which, by discharging, may also act to intensify and prolong the effects of affective stimulation.

The critical system on the other hand besides the control which, within certain limits of stimulation, it exerts upon the affective system, supplies, in the intact animal, the physiological basis for postural recognition and spatial orientation which in turn supply the basis for selective adjustments with all that these imply in the way of psychic development. The affective system when released by injury or disease from the modifying control of the critical system tends to function maximally, i. e., on the all or none principle but when it functions in coöperation with the critical system, as normally it always does within certain ranges of affective stimulation, the response tends to be graded, i. e., proportioned to the intensity of the stimulus or so to speak to the needs of the situation. In the spinal animal the objective reactions to affective and critical stimulation are seen in the flexion reflex and extensor thrust respectively although even in the spinal preparation the critical mechanisms exert some controlling influence upon the affective because the response to affective stimulation is at times manifestly graded. In the decerebrate animal the economizing influence of the critical system manifests itself in a striking manner. In such preparations the plastic tonus of the antigravity muscles is main-

tained at a minimum of metabolic outlay. Compare Langelaan (13) and Sherrington (14). The relations, demonstrated in the present studies, of the proprioceptive system to the cardio-inhibitory mechanism seem but a further instance of the integration of the economizing mechanisms controlled by the critical system. In the intact animal the *instinctive* (imperative) modes of protective reaction rest fundamentally upon the affective system whereas the cognitive (selective) modes rest fundamentally upon the critical system which, by inhibiting the affective system, and substituting the graded for the all or none response, acts not only as a *conservator* of physical energy but, by rendering it possible for the animal to stand still and investigate instead of running away or hastily withdrawing the part, supplies the first essential requisite for orientation (stimulus evaluation) which in turn is the sine qua non of cognitive (selective) defense reactions and indeed of all education and training. With the aid of the projicient receptors (visual, auditory, etc.), the critical system enables the individual not only to avoid injury without risk of actual noxious contact but it also supplies the means for evaluating the situation at a safe distance and so of regulating the expenditure of somatic and psychic energy according to the needs of the situation. From this it is but a step to the further elaboration which through comparison, judgment, memory, etc., affords protection in its widest scope and supplies the security necessary for higher psychic integration. The cardio-inhibitory mechanism driven on the afferent side by the critical system seems, therefore, to be an energy conserving mechanism which normally functions side by side with the antagonistic accelerator mechanism driven on the afferent side by the affective system. In the startled animal the accelerator mechanism functionally overshadows the inhibitor mechanism which may nevertheless continue to exert its energy conserving and sustaining functions. Compare the pupil dilator and constrictor mechanisms. On the other hand the influence of the critical system as a conservator of energy manifests itself not only in the enhanced endurance of the seasoned athlete but also in his poise and ease of movement all of which so largely depend upon education (training).

The results obtained in these studies seem also to point the way for better mechanistic interpretation in "vegetative neurology." At first sight it may seem that phenomena such as paradoxical cardiac inhibition which cannot readily be induced by lesion of the somatic nerves whilst both vagi are intact would have little, if any, significance for the clinician. However, the deleterious products of disease, injury, etc., like all toxins, exogenous or endogenous, may, and frequently do, affect the afferent and efferent nerve paths of both systems, e.g., accelerator and inhibitor, and on both sides of the body simultaneously. These and other facts, e.g., those pertaining to selective affinity, local immunity, focal infections.

etc., furnish the beginnings for a more secure mechanistic interpretation in some of the phenomena attending cardiac disorders, e.g., extra-systole, tachycardia, bradycardia, fibrillation and even angina pectoris, in any one of which, for example, the lesion without necessarily involving the cardiac structures directly may predominantly affect one or other of the governing systems, accelerator or inhibitor, on the afferent or efferent side. And as the critical system is more vulnerable than the affective (9) and recovers full function after injury or disease more slowly than the affective system it is not surprising to find that phenomena resembling in nature and mechanism those of paradoxical cardiac inhibition may be encountered as a consequence of the prolonged bodily inactivity incidental to protracted illness.

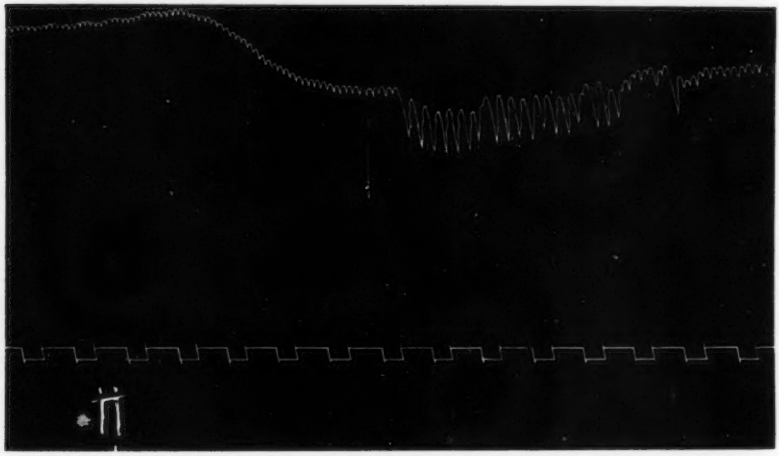

Fig. 12. Cat 6 lbs., 8th day after section of left vago-sympathetic and destruction of right otic labyrinth. Pilocarpine hydrochloride gr. $\frac{1}{100}$ at ii. Paradoxical effects.

In such conditions the intracardiac inhibitor mechanisms become hypersensitized just as they do in animals after section of the tendons of the antigravity muscles. Indeed the author has seen extrasystoles, etc., and even well-marked anginoid attacks closely resembling paradoxical cardiac inhibition evoked in athletic individuals of middle age by the too abrupt resumption of active exercise (moderately brisk walking) one or two days after a three weeks' period of recumbency. In such cases the prolonged inactivity of the antigravity muscles and other postural mechanisms, e.g., blood vessels, etc., causes suspension of function in the proprioceptive mechanisms conditioning the normal efferent flow of inhibitory impulses over the vagi with consequent hypersensitization of the intracardiac inhibitory mechanisms the immediate excitant of the angi-

noid (paradoxical inhibitory) phenomena being, as certain facts seems to indicate, the products of metabolism (humors) incidental to the unaccustomed active functioning of the over-rested nerves, afferent and efferent, mediating cardio-inhibitor function. Compare Byrne (1) who found that after double vagotomy paradoxical inhibitory phenomena were readily elicited by extracts of fatigued vagus nerve made after the manner of Brinkman, of von Dam Jendrassik, and of Hamburger. The author realizes, however, that the findings in the present studies present a problem which calls for clinical investigation on its own account.

CONCLUSIONS

1. After lesions of the proprioceptive system a short period of incubation occurs prior to the development of paradoxical sensitization of the intracardiac vagal mechanisms.

2 The bilateral distribution, central and peripheral, of the afferent and efferent nerves serving the cardio-inhibitory mechanism renders it difficult or impossible to obtain frank paradoxical effects with both vagi intact except from lesions in sites in which marked condensations of proprioceptive mechanisms occur, e.g., the otic labyrinth, upper spinal cord, etc.

3. Normally the heart movements, like those of the iris, are governed by two separate sets of reciprocally related neural mechanisms, viz., *a*, the inhibitor set served on the efferent side by the parasympathetic (vagal) system driven on the afferent side by the critical (proprioceptive) system; and *b*, the accelerator set served on the efferent side by the sympathetic system driven on the afferent side by the affective system. Each system functions fractionally and as a whole in a coöperation of antagonism with the other in controlling cardiac movements.

4. The accelerator (affectivo-sympathetic) system preponderately governs systole and thereby contributes to the maintenance of adequate systemic blood pressure whilst the inhibitory system preponderately governs diastole which to some extent seems to be an active process.

5. The present studies seem to support, with some modification, the hypothesis of the duality of striated muscle in which antagonistic effector mechanisms, served by recriprocally related neural mechanisms coöperate to effect and sustain movement and posture.

6. The kinetic element is mediated by the anisotropic discs served on the efferent side by the cerebro-spinal fibers driven on the afferent side by the affective system and its equivalent in the lower sensory-psychic domain (gross emotional sphere) whilst the tonus element is mediated by the sarcoplasm served on the efferent side by the parasympathetic system driven on the afferent side by the critical (proprioceptive) system and its equivalent in the higher sensory-psychic domain (election, will).

7. In striated muscle throughout the entire body the kinetic and tonus elements function side by side in a coöperation of antagonism which is the counterpart, on the efferent side, of that under which the two great afferent systems (affective and critical) function.

8. In the heart the effectors mediating the kinetic or tonus element seem to have but one source of efferent nerve supply, viz., affectivo-sympathetic for the kinetic element and critico-parasympathetic for the tonus (inhibitory) element. It is possible, however, that like the pupillary constrictor and dilator mechanisms the kinetic effectors receive some critico-parasympathetic and the tonus effectors some affectivo-sympathetic supply.

9. In contrast with the accelerator (affectivo-sympathetic) mechanism, which tends to function on the all or none principle, the cardio-inhibitor (critico-parasympathetic) mechanism follows the principle of graded response and seems to be one of the many instances of the economizing effects of critical integration.

10. In the fully integrated animal the two great afferent systems, affective and critical, are the primary drivers and regulators of all our activities (motor, inhibitory and secretory) in so far as these pertain directly or indirectly to environmental adjustment.

11. The paradoxical phenomena induced in the heart and iris as the result of injury or suspension of function in the afferent and efferent nerves, somatic and visceral (including cerebral), furnishes the beginning in a new direction for better mechanistic interpretation in visceral disease (vegetative neurology).

12. The coöperation of antagonism, under which the affectivo-sympathetic and critico-parasympathetic systems function throughout the body, acts fundamentally not only as a conservator of somatic and psychic energy (graded response in protective reactions) but also furnishes the means, in the first instance, of environmental orientation (stimulus evaluation) which is the *sine qua non* not only of cognitive (selective, reasoned) defense reactions but also of sensory-psychic integration.

13. After prolonged inactivity of the antigravity muscles such as that incidental to long illness necessitating recumbency the sudden resumption of active exercise may evoke extrasystoles, etc., or even anginoid attacks which in nature and mechanism seem to be identical with paradoxical cardiac inhibition.

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STUDIES ON THE CLASPING REFLEX IN AMPHIBIA

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Although the literature on this subject is old, little seems to be known and some of that incorrectly. In 1887, the curiosity of Tarchanoff (7) was aroused as to the mechanisms concerned in the sexual desire and the clasping reflex in the frog. He found that when the male was clasping the female, no stimulation or injury to the male interfered with the clasping except injury to the brain itself. In corroboration of Goltz's (1) earlier experiments, he found that removal of the enlarged testes in the breeding season had no influence upon the male clasping reflex, if the testes were removed while the clasping was being performed. Goltz found that stroking the skin over the sternum easily evoked the clasping reflex.

The most important work in this field was done by E. Steinach. In his first paper in 1894 (6a), he showed that sexual behavior in the male was independent of the seminal vesicles and that castration in Rana temporaria two months prior to the breeding season greatly diminished the sexual desire during the breeding season for they never showed spontaneous clasping of female and when placed with legs around female only manitained position for a few minutes compared to the several days' time which the uncastrated male holds on to the female. In 1910, he stated (6b) as a result of 95 castrations that if sufficient time were permitted to elapse all tendency to clasping was lost. Upon these animals he performed numerous experiments. He injected testicular substance removed from animals showing vigorous clasping reflex (in the dorsal lymph sac) and observed in 88 per cent of the animals a return of the reflex as elicited by stroking the sternum with the finger. He found further that when brain substance was thus injected the results were also positive, due, as he thought, to the storage of the testis hormone there, normally, the seat of psychic sexual activity. He concluded from these experiments that the testes produce a specific hormone which has a selective effect upon "clasping centre" in the nervous system and thus upon the clasping reflex. Thus, according to Steinach, the clasping reflex is due to a specific hormone secreted by the testes.

Harms (2) and Meisenheimer (5) found that ovarian extract had the same effect in producing the return of the clasping reflex in castrated

males, but Steinach, who found the same, claimed that the results were much less positive.

Steinach (6a), (6b) and Langhans (4) also studied the nervous control of the clasping reflex. They "decapitated" by transection at the level of the midbrain and used the cautery in destruction of the forebrain and concluded that there are inhibitory centers in the corpora bigemina and cerebellum, for after one or two days following the removal of the forebrain and corpora bigemina the clasping reflex is increased in intensity.

They found that when the sex pad was cocainized or surgically removed the clasping reflex was no longer obtainable by stroking the sternal skin, whereas mere stimulation of the sex pads normally elicits the reflex, and they claimed therefore that Goltz's conclusion that stroking the external skin produced the reflex was incorrect.

EXPERIMENTAL. Toward the end of April we began a series of experiments on the clasping reflex of the leopard frog, Rana pipiens.

Normal male frogs were used. They all showed vigorous clasping by the method of Goltz, that is, when the sternal skin was stroked with the finger.

The sex pad of one leg was removed and the sternal skin stroked, a slight reflex resulting. After twenty minutes the normal reflex was present without any observable alteration or diminution. The experiment was repeated upon several animals with exactly the same result.

The sex pads were cocainized by injection of 5 per cent cocaine solution into both pads. The reflex was present without impairment after a few minutes and was not abolished during the observation period of several hours. These experiments were repeated upon animals by placing a sponge soaked in 5 per cent cocaine over pad for one hour. Reflex response in all cases quite normal.

The pad of numerous normal animals was stimulated by touching the pad with instruments or the fingers and with the faradic current. In every case, where there were any movements of the leg they were extension and never in any respect resembled clasping.

The faradic current was applied to the skin of normal animals with the bipolar electrode. The skin was entirely negative at every place except directly over the sternum and upon the ventral aspect of the front legs. The experiments were repeated stroking the skin with the finger with exactly the same results.

The abdomen of animals of both sexes was opened and the viscera stimulated, testes, ovaries, etc. All were uniformly negative.

Pithing of the cord abolished the reflex. Several animals were placed in ice water for 24 hours. After this time the reflex was present although it was sluggish and less intense.

The forebrain, diencephalon and corpora bigemina were removed from normal animals. These animals were observed for over a week. They

were never seen to show any tendency to spontaneous clasping. When the sternal skin was stroked, clasping was elicited which was normal in occurrence and in relaxation. Stimulation of the pads gave uniformly negative results.

The brain was exposed in numerous animals and stimulated with faradic current and bipolar electrodes. When a weak current was used no clasping response was elicited until the mid-medullar region was reached. When this region was stimulated, the clasping position was taken and maintained as long as stimulation was continued. When either side of this mid-medullar region was stimulated the response was bilateral but seemed to be somewhat stronger on the homolateral side.

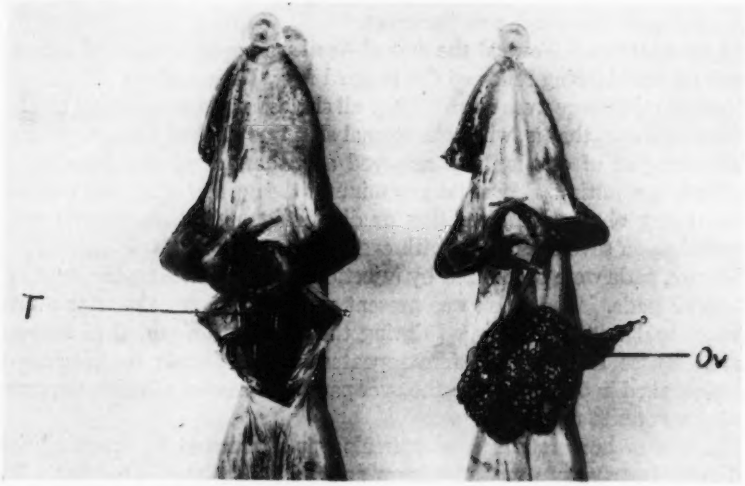

Fig. 1. Male and female frogs showing clasping reflex produced by section in lower medulla. *t* = testes; *ov* = ovaries.

The brain of normal animals was exposed and sectioned at various levels. When the section was anywhere above the mid-medullar region, the reflex clasping was obtained normally by stimulation of the sternal skin. There was never any tendency toward spontaneous clasping and the clasping relaxed as quickly as normally.

When the section was made through the mid-medullar region or below it, the animals often took the clasping position spontaneously and maintained it for from several minutes to hours. When the finger was inserted under the clasped legs the animal clasped it with great vigor and the animals could thus be held in the air for several minutes.

The behavior was the same when the section was made at any level as low as just above the exit of the nerves supplying the fore legs. Any section below the level abolished the reflex.

When the lower medulla was hemisected, faradic stimulation of "centre" produced bilateral clasping response somewhat weaker on the same side.

The skin was removed from the inside of one fore leg in several animals and the lower medulla cut across. After a number of hours (one to twelve) the reflex was not obtainable by stroking the bare muscle but a normal and bilateral reflex resulted from stroking the skin of the other leg.

These experiments were repeated upon female frogs with exactly the same results in every case except, of course, no sex pads being present the experiments upon them were omitted. Autopsy was performed upon numerous of these animals and normal ovaries and oviducts were found and in no case, testes.

As with the clasping reflex so with the croaking reflex male and female behaved exactly alike.

The clasping reflex was studied on the newt Diemyctylus torosus from Oregon. When the skin of the ventral surface of the body was stroked with the finger the animal strongly clasped it with its four legs and wound its tail around it.

The mid medullar region was stimulated by bipolar faradic current and the complete reflex obtained. Section of the cord, in mid thoracic region, separated the units so that the hind legs and tail responded by clasping when the skin over the pelvic girdle was stroked and did not respond to medullar stimulation or to stroking of skin over the pectoral girdle. The two series showed the clasping reflex indistinguishably.

Discussion. It will be recalled that Goltz considered the clasping reflex to be the response to stimulation of the sternal skin, whereas Steinach and Langhans contended that the stimulation was dependent upon the sex pad and had to be made upon them. Our results appear to agree conclusively with Goltz. For with the sex pads completely removed, the clasping reflex is present unaltered and cannot in any case be obtained by stimulation of the pads of normal animals. It is possible that the difference between Goltz's and our results on the one hand and Steinach and Langhans' on the other is to be explained on the basis of the different species of frog. We further corroborated Goltz's contention that the only excitable area for eliciting the reflex is the sternal skin, the entire viscera and skin being negative elsewhere, that is, except the inner surface of the front legs which apparently had not been noticed before. We consider this sensory area as essential for the reflex as the sternal skin, for stimulation of it on either side gives bilateral response of the same vigor as when elicited from the sternal skin.

We were unable to confirm Steinach and Langhans' statement that the midbrain and cerebellum contain an inhibitory center, the removal of which caused spontaneous clasping. On the contrary, we found that elimination of the entire brain above the mid-medulla had absolutely no obvious influence upon the clasping reflex either spontaneous or reflexly.

The mid-medulla region appears to be a reflex "centre," for *a*, section on stimulation above it has no effect on the reflex; *b*, section below it causes spontaneous clasping and greatly exaggerates the intensity and duration of the reflex when elicited by stimulation of the skin; *c*, hemisection below the region does not influence the clasping caused by stimulation of the excitable area except to slightly weaken it upon that side.

We confirmed the old observation that the clasping is a reflex and not a mere muscular response for stimulation of the muscle beneath the excitable skin does not evoke the clasping, and pithing the cord abolishes it.

We were much surprised to observe that the female behaves exactly as the male in the clasping behavior. Thus, it is obvious that the clasping behavior is not dependent upon the *testes* or their secretions. This observation may have some bearing upon the conclusions of Steinach (6), Meisenheimer (5) and Harms (2) that ovarian extract caused a return of the clasping reflex in castrated males in the breeding season. Therefore, if a secretion from the gonads is necessary for the clasping reflex, it is bisexual and *non-sexually specific*. The large number of these females, the age, and the absence of testis-like tissue at autopsy assures us that these could not possibly be Pflügerian hermaphrodites.

It was shown recently by Koppanyi (3) and others independently that the Spanish newt, Pleurodeles Waltli, has the clasping reflex similar to the Anura. The Diemyctylus torosus having sex pads and breeding in the water like Pleurodeles waltli and unlike other urodeles, we were led to expect that it might also show this reflex. Therefore, we believe that the clasping reflex of amphibians is dependent merely upon the breeding habits and is not peculiar to the anura.

SUMMARY

1. The sex pads are not necessary for the performance of the clasping reflex and are not associated with it in a sensory way.

2. The skin upon the inner surfaces of the front legs as well as that over the sternum when stimulated evokes the clasping reflex, whereas no other part of the body does, except the direct nerve pathways.

3. An excitable area corresponding to a reflex "centre" for the clasping act is located in the mid-medullar region.

4. The clasping reflex of the male is indistinguishable from that of the female.

5. The newt Diemyctylus torosus shows a clasping reflex similar to the frog.

6. The clasping reflex is characteristic of amphibia and is dependent merely upon the breeding habits.

It is with pleasure that we acknowledge our indebtedness to Doctor Carlson, whose criticism and help have made this work possible.

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SECRETIN

VI. Its Influence on the Antibodies of the Blood: Agglutinin

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The changes in the corpuscle content of the blood and the general improvement which follows the treatment of rabbits by subcutaneous injections of secretin have been recorded elsewhere (1). In connection with this work we have noted that wounds accidentally inflicted in various ways upon our secretin-injected animals healed more rapidly than similar wounds in animals not receiving secretin. This result might be the effect of the nutritional improvement alone, but there is a possibility that it is contributed to by an increased antibody production. We have sought to test the latter possibility by quantitative investigation of various types of antibodies.

An agglutinable typhoid culture was obtained according to the method of the Department of Pathology of Oxford University (2), as follows:

> The bacillus is grown for 24 hours at 37°C. in ordinary veal peptone bouillon in large Erlenmeyer flasks partly filled (1 litre of bouillon in a one and a half litre flask).
>
> Before use the flasks of bouillon are sterilized in the autoclave at 115°C. for not more than 15 minutes, and are then tested for sterility by incubation at 37°C. for 48 to 72 hours.
>
> They are inoculated with a few drops each from a 20 to 24 hour old bouillon culture of the bacillus (B. typhosus).
>
> The culture used should be one which has been subcultivated daily in bouillon for one or two weeks (or longer). This continued subcultivation has the effect of increasing its agglutinability and diminishing any tendency to spontaneous agglutination.
>
> At the end of 20 to 24 hours' growth at 37°C. the flasks are well shaken and to each is added 0.1 per cent (1 cc. per litre) of commercial (40 per cent) formalin. They are again shaken and placed in a cold chamber in the dark at about 2°C.
>
> At intervals on the same day and on subsequent days for 4 or 5 days the flasks are again thoroughly shaken and replaced at once in the cold chamber.
>
> After 3 or 4 days they will be found to be absolutely sterilized. Should it happen that the bacterial suspension is not entirely homogeneous it may be shaken for some hours in a mechanical shaker, or may finally be filtered through sterile cotton wool.

By this method we prepared at one time a suspension of killed typhoid bacilli in sufficient quantity to use for all the intraperitoneal injections and all the agglutinin determinations.

Twelve adult healthy rabbits which had never been experimented upon were selected, numbered and weighed. Each rabbit was bled from an ear vein and a determination made of the power of the blood to agglutinate B. typhosus. One week later the rabbits were re-weighed and divided into two groups of as nearly equal weight as possible. The average weight of the secretin group was 2,257 grams and of the control group 2,230 grams. On this day, counted as the first day of the experiment, every rabbit received an intraperitoneal injection of 2 cc. of the suspension of killed typhoid bacilli. Each rabbit of the secretin group was also given subcutaneously a dose of secretin preparation (dried acid extract), prepared as described previously (3), in the proportion of 15 mgm. of the preparation for each kilogram of body weight. This was dissolved in 0.9 per cent saline and heated to body temperature at the time of injection. The secretin injections were repeated daily for twenty-one days. Seven days after the first injection of typhoid bacilli all the rabbits were given an additional 4 cc. of the sterile culture of B. typhosus intraperitoneally. The agglutinating strength of the blood of every rabbit was determined on the fifth, eighth, twelfth, fifteenth, nineteenth and twenty-second days. All the animals were weighed once a week. The average changes in weight were the same in both groups; both declined slightly in the second week, and gained in the third week so that the final averages were, for the secretin group 2,315 grams, for the control group 2,332 grams.

For all the titrations the test tubes and pipettes used were washed, rinsed in distilled water and dried upside down in a hot oven. The rabbits were bled, the blood kept on ice over night and the serum pipetted off.

In the preliminary titration of agglutinin before injection of the bacilli the procedure was as follows: Seven tubes were provided for each rabbit and in each of these tubes was placed 1 cc. of sterile physiological saline solution. To the first tube 1 cc. of the rabbit's blood serum was added and mixed with the saline by means of the pipette. One cubic centimeter of this mixture was placed in tube 2 and mixed with the saline. One cubic centimeter from tube 2 was then transferred to tube 3 and so on until six tubes had been used. One cubic centimeter was removed from tube 6 and discarded. In another tube, called A, was then placed 1 cc. of serum but no saline. One cubic centimeter of the sterile typhoid culture was next added to each tube, the contents mixed by shaking and the tubes plugged with absorbent cotton. The mixtures were incubated at 50°C. to 55°C. for 2 hours, removed from the oven and allowed to stand at room temperature for 20 minutes. In reading agglutination we adopted the usual standard, i.e., the highest dilution of the serum showing total agglutination. The readings were made by artificial light against a dark background. The dilutions of serum were

	TUBE A	TUBE 1	TUBE 2	TUBE 3	TUBE 4	TUBE 5	TUBE 6	TUBE 7
Dilution.....	1:2	1:4	1:8	1:16	1:32	1:64	1:128	0

In no tube was there complete agglutination. In other words, the blood serum of none of our rabbits was able to fully agglutinate B. typhosus even when the dilution was only one part in two.

TABLE 1

Highest dilution of blood serum producing complete agglutination

ANIMAL	PRELIMINARY	5TH DAY	8TH DAY	12TH DAY	15TH DAY	19TH DAY	22D DAY
			Secretin group				
20	Incomplete at 2	Incomplete at 20	600	1250	1400	1800	1400
28	Incomplete at 2	Incomplete at 20	700	1500	2800	3600	3600
31	Incomplete at 2	Incomplete at 20	200	1500	2800	4000	4400
36	Incomplete at 2	Incomplete at 20	400	1500	3200	3400	3600
38	Incomplete at 2	Incomplete at 20	700	1250	2600	3400	3600
41	Incomplete at 2	Incomplete at 20	Incomplete at 50	100	100	200	200
Average..	Incomplete at 2	Incomplete at 20	433	1183	2150	2733	2800
			Control group				
27	Incomplete at 2	Incomplete at 20	500	1000	1400	3000	2400
30	Incomplete at 2	Incomplete at 20	500	1500	2800	3400	3000
32	Incomplete at 2	Incomplete at 20	100	1500	3000	3200	3400
37	Incomplete at 2	Incomplete at 20	300	1250	2600	3000	3000
39	Incomplete at 2	Incomplete at 20	300	1000	1200	2400	2400
40	Incomplete at 2	Incomplete at 20	Incomplete at 50	Incomplete at 50	100	100	100
Average..	Incomplete at 2	Incomplete at 20	283	1041	1850	2516	2383

In titrating agglutinin subsequent to the intraperitoneal administration of a suspension of dead typhoid bacilli the method was the same as described, the only difference being the use of higher dilutions. In each of these six determinations the series of dilutions prepared for any individual rabbit was determined by the previous reading for that animal. The results of these observations as well as the preliminary finding are collected in table 1.

On February 19, the first typhoid injection having been made on the 15th, there was no tube showing complete agglutination though agglutination was apparent in every rabbit. Rabbit 36 showed some agglutination even when the blood serum was diluted 1 to 450 and the readings at which a trace of agglutination could be detected ranged downward from this to a dilution of 1 to 200. The lowest dilution used on this day was 1 to 20 and even then, as previously stated, agglutination was not complete. On the 22nd of February the lowest dilution made was 1 to 50 and the blood of two rabbits, 40 and 41, still failed to give complete agglutination. For some reason these rabbits showed comparatively feeble agglutinating power throughout, though apparently in good health.

CONCLUSIONS

1. The agglutinating power of the blood of rabbits receiving secretin averaged higher than that of rabbits that were not receiving secretin.

2. The average agglutinating power of the secretin rabbits was 53.00 per cent higher at the end of the first week than the control rabbits, 16.21 per cent higher at the end of the second week and 17.49 per cent higher at the end of the third week.

We wish to acknowledge our indebtedness to Dr. R. M. Shaw, of the Department of Bacteriology, for advice and assistance in the carrying out of this experiment.

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SECRETIN

VII. Its Influence on the Antibodies of the Blood: Complement

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At the same time that agglutinin determinations were being made, as described in the preceding article of this group (1), a portion of the blood serum available from the experimental rabbits was used to estimate the relative amounts of complement. For this purpose a suspension of washed sheep's corpuscles was prepared by drawing about 12 cc. of blood from a sheep directly into 18 cc. of sodium citrate solution of a strength of 1 per cent in 0.85 per cent sodium chloride solution contained in a graduated centrifuge tube. This was centrifuged thoroughly. The corpuscles were then washed and a 1.25 per cent suspension in normal saline solution made. Standard amboceptor, which was used throughout the experiment, was provided next. The amount of this to be used was determined by finding how much was required to hemolyze 0.5 cc. of the suspension of sheep's corpuscles with a known complement, guinea pig serum, added. The amboceptor was rabbit serum diluted 150 times, and the quantity required fixed at 0.2 cc.

In making the first determination ten test tubes for each rabbit were set up and in the first tube 1 cc. of a 1 to 10 dilution of the blood serum to be tested was placed, in the second tube 1 cc. of a 1 to 20 dilution, and so on on up to 1 to 100. To each tube was added a mixture of blood corpuscles (1.25 per cent suspension) 0.5 cc., amboceptor 0.2 cc., and normal saline 0.8 cc., which had been incubated for five minutes at 37°C. The contents of the tubes were mixed and incubated in a water bath for one hour at 37°C. In each set the tube of highest dilution showing complete hemolysis was noted.

Two determinations of complement were made before the injection of 2 cc. of a suspension of killed B. typhosus into the peritoneal cavity of each rabbit. The method by which this suspension of dead bacilli was prepared is described in connection with the determination of agglutinating power (1). Beginning on the day that the typhoid bacilli were injected and continuing for twenty-one days, each rabbit in the secretin group received daily a subcutaneous injection of a secretin preparation (2) in the proportion of 15 mgm. per kilogram of body weight. This was dissolved

in 0.9 per cent saline solution, 10 mgm. to 1 cc., and heated to body temperature at the time of injection. A second intraperitoneal injection of the killed typhoid bacilli was made seven days after the first. The dose on this occasion was 4 cc. per rabbit.

Complement determinations were made twice weekly for three weeks following the first typhoid injection. It was soon seen that dilutions

TABLE 1

Highest dilution of blood serum (complement) producing complete hemolysis

ANIMAL	PRELIMINARY		5TH DAY	8TH DAY	12TH DAY	15TH DAY	19TH DAY	22D DAY
Secretin group								
20	18	18	28	26	25	18	18	16
28	22	18	18	16	26	18	22	16
31	24	18	28	38	29	26	26	28
36	28	28	24	16	28	26	24	18
38	16	22	26	28	27	26	26	26
41	24	38	28	16	26	24	27	24
Average...	22.8		25.3	23.3	26.8	23.0	23.8	21.3
Control group								
27	18	18	16	12	18	18	16	18
30	22	22	24	26	26	24	16	22
32	24	24	26	28	28	28	26	24
37	26	26	26	28	28	24	22	24
39	18	24	18	24	24	26	18	16
40	26	28	28	36	38	28	20	22
Average...	22.9		23.0	25.6	27.0	24.6	19.6	21.0

ranging up to 1 to 60 would be sufficient and the higher dilutions were dispensed with. Furthermore, a system was devised by which the degree of hemolysis at intermediate points could be indicated and the figures given in the accompanying table are dilutions of the blood sera tested which caused complete hemolysis of the sheep's corpuscles.

CONCLUSIONS

1. The complement of rabbit's blood was estimated by its hemolytic action on washed sheep's corpuscles.

2. Daily subcutaneous administration of a secretin preparation for three weeks did not affect the complement content of the blood of the rabbits used.

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SECRETIN

VIII. Its Influence on the Antibodies of the Blood: Hemolytic Amboceptor

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In order to determine what influence, if any, secretin exerts on the amount of amboceptor in the blood, ten apparently healthy rabbits were selected and divided into two groups. None of these rabbits had been used for any other experimental procedure. They were numbered for identification and weighed. One group, known as the secretin group, received daily injections of secretin, 15 mgm. of a dried acid extract per kilogram of body weight; the other group was designated the control group. The secretin used was prepared from the dog by the method described previously (1) and at the time of injection was dissolved in normal saline solution, heated to 37°C. and injected subcutaneously. The secretin preparation used in this and all other experiments with secretin was tested physiologically and proven active.

The rabbits in the control group were numbered 21, 25, 33, 34 and 35. On the fourteenth day number 21, a male, died and on the twenty-seventh day number 33, also a male, died. The rabbits in the experimental group were numbered 22, 23, 24, 26 and 29. On the fifteenth day number 22, a female, died. The average weight of the five test rabbits at the beginning of the experiment was 2,394 grams. Of the four rabbits of this group that survived the average weight at the beginning was 2,440 grams and at the end 2,449 grams. Daily injections of secretin did not affect the body weight of rabbits that were well nourished when the experiment began and that were given a full diet during the experiment. The average weight of the five control rabbits at the beginning was 2,198 grams; of the three that survived the average weight at the beginning was 2,289 grams and at the end 2,201 grams.

The experiment covered a period of twenty-nine days. On the first day 4 cc. of blood were withdrawn from the ear vein of each rabbit and 10 cc. of washed sheep's corpuscles in 10 cc. of 0.9 per cent sodium chloride solution injected intraperitoneally. Every day thereafter for twenty-eight days each of the rabbits in the experimental group received a subcutaneous

injection of secretin. Twice each week samples of blood were taken from the ear veins of all the rabbits. Altogether nine specimens of blood were collected from each rabbit. The hemolytic activity of each of these was determined by the following method.

The serum of each specimen of blood was pipetted off after coagulation had taken place, heated to 56°C. for thirty minutes and kept at a temperature of 10°C. until the tests were made. At that time three dilutions of the serum were made, 1 to 20, 1 to 40 and 1 to 80. One cubic centimeter of 0.9 per cent sodium chloride solution was placed in each of a series of thirty test tubes and blood serum of each of the three dilutions added in ten different amounts, 1.0 cc., 0.8 cc., 0.6 cc., 0.5 cc., 0.4 cc., 0.3 cc., 0.2

TABLE 1

Highest dilution of blood serum (amboceptor) producing complete hemolysis

ANIMAL	INITIAL	4TH DAY	7TH DAY	11TH DAY	14TH DAY	18TH DAY	21ST DAY	25TH DAY	28TH DAY
				Secretin group					
22	3.0	25.0	160.0	800.0					
23	6.0	66.6	133.3	1600.0	133.3	66.6	66.6	133.3	40.0
24	8.0	100.0	160.0	600.0	106.4	53.2	100.0	133.3	40.0
26	10.0	18.0	68.0	80.0	68.0	38.0	38.0	25.0	18.0
29	6.0	17.0	200.0	266.6	106.4	80.0	80.0	53.2	40.0
Average....	6.6	45.3	144.2	669.3	103.5	59.4	71.1	86.2	34.5
				Control group					
21	3.0	66.6	133.3	600.0					
25	3.0	25.0	400.0	800.0	106.4	300.0	133.3	200.0	53.2
33	0	150.0	1600.0	1600.0	600.0	300.0	200.0	133.3	
34	0	50.0	800.0	800.0	400.0	300.0	300.0	300.0	133.3
35	0	33.3	800.0	800.0	400.0	200.0	133.3	100.0	53.2
Average....	1.2	64.9	746.6	920.0	376.6	275.0	191.6	183.3	79.9

cc., 0.15 cc., 0.1 cc. and 0.05 cc. This gave a range of dilutions from 1 to 20 to 1 to 1600 of amboceptor added. This was followed by 0.5 cc. of a 2.5 per cent suspension of washed sheep's corpuscles in normal saline solution. The complement, guinea pig serum diluted 1 to 10, was next added. The amount in each case was 0.15 cc., determined by titration against a known amboceptor of a titer of 6000. Finally a sufficient quantity of normal saline solution was added to bring the total bulk to 2.5 cc. These were thoroughly mixed and incubated at 37°C. for thirty minutes. The highest dilution of amboceptor that gave complete hemolysis in each series was noted. All of the tests were made at one time using the same complement and the same preparation of sheep's corpuscles.

The results for all the bloods are collected in table 1. The only departure from this method was in testing the initial bloods, those taken before the intraperitoneal injection of sheep's corpuscles. In these normal saline was added to give a dilution of the serum of 1 in 5.

CONCLUSIONS

1. The amount of amboceptor contained in rabbit's blood was estimated by its hemolytic action on washed sheep's corpuscles.

2. In the blood of the rabbits receiving secretin the hemolytic power against washed sheep's corpuscles increased on the average approximately one hundred times.

3. In the blood of the rabbits that did not receive secretin the hemolytic power increased eight hundred times.

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THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON METABOLISM

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The debate on the question of the influence which changes in environmental temperature exert on basal metabolism is of very old standing. Rubner differentiated two regulatory processes concerned with the maintenance of body temperature: 1, a chemical mechanism of regulation, operative at low temperatures, and 2, a physical mechanism of regulation, operative at high outside temperatures. The former increases the heat production through the stimulation of metabolic activity in the organism, while the latter reduces the total heat content of the body by increased dissipation from the skin through perspiration. Richter believes that the chemical regulation becomes operative once more at very high outside temperatures, the processes of combustion in the organism becoming more limited under these circumstances. It is not the purpose here to survey the somewhat extensive literature bearing on this subject, especially as this has been discussed in various reviews of the general topic of metabolism. I merely wish to emphasize the two principal viewpoints which compete for predominance in the interpretation of results obtained in experimental studies of the metabolism under different environmental temperatures, namely, that of the Rubner school, which assumes the existence of an independent chemical regulatory process, the cold *per se* stimulating the cells to greater activity, and that of the Pflüger school, which regards the variations in metabolism as being regulated through nervous impulses arising from peripheral stimulation and causing reflexly a muscular response. On this last view shivering induced by low temperature would be the primary cause responsible for the changes in the rate of metabolism. Although the significance of muscular activity in the metabolic response of the organism to temperature variations has thus been very early recognized, little attempt has been made even in studies of recent date to control this variable factor of behavior by some objective means. Johansson in a very thorough and exhaustive investigation on human subjects came to the conclusion that no chemical temperature regulation exists without the direct response from the muscular system. Murschhauser, who studied the effect of extreme temperatures (5 and 35°)

on guinea pigs, found that the carbon dioxide production and oxygen consumption were 45 and 49 per cent respectively higher in the cold and contends that a definite relationship between the body surface and the rate of metabolism is maintained at all temperatures. Although Murschhauser's work has appeared in relatively recent years, he is obviously not fully aware of the magnitude of the influence which muscular exertion has been shown to have on metabolism. In a general way he claims that his animals were *very quiet* during the experments, but at the same time he observes that "sie wechseln wohl hin und wider ihre Stellung, um dann für längere Zeit in derselben zu verharren." This quotation is sufficient to show how little appreciation there is of the influence which even small movements do exert upon the metabolic rate and, in the absence of an objective measure of the animal's reactions, metabolic studies are entirely unreliable. Within this past year another investigation dealing with this problem has appeared, to which the same criticism may be applied with equal force. Goto, experimenting with rats, finds a definite relationship between the external temperature and the metabolic activity, the latter diminishing as the environmental temperature increases reaching a minimum at a certain optimum temperature (about 28°C.). Goto seems to neglect entirely the possible influence of factors other than that of the temperature change, maintaining that the metabolism rises in a definite proportion for every degree of temperature difference. It may be well, in view of these claims of an independent influence of temperature on cellular metabolism, to point out that even in cold blooded animals this is probably not a simple matter of temperature effect. In these organisms there is a more or less close and direct relationship between the metabolic rate and the environmental temperature, since the *internal* temperature of the animal actually fluctuates with the variations of the *outside* temperature. Nevertheless, there is no evidence that the metabolic reaction even in these animals follows the same simple rule observed in ordinary chemical reactions, where the rate of reaction increases in a definite manner for every 10°C. difference. The recent studies of Dirken on frogs demonstrate that the temperature coefficient of metabolism for a 10° difference in temperature varies very widely, depending upon the range of temperature (10 to 30°) studied.

These recent experiments purporting to establish an experimental basis for the theory of chemical temperature regulation prompt me to publish the results of experiments which I carried out on dogs several years ago. In these studies the attempt has been made to obtain an objective record of the animal's behavior in the course of an experiment and to eliminate such experiments where the results were influenced by muscular exertion. Another objective criterion likewise used in these studies will be discussed presently. All of these objective

controls permit an interpretation of the variations of metabolic activity resulting under different temperatures for which the other reported studies give no basis whatever.

The experiments were performed on dogs, usually 24 hours after their last meal. The animals were thoroughly used to the experimental procedure. The experiments were made in a Benedict closed circuit respiration apparatus designed for small animals. The dog was placed in a galvanized wire cage which rested along one side on a knife edge fixed to the bottom of the apparatus and was suspended at the opposite side by a very flexible spring. This arrangement imparted to the cage great mobility, the least motion on the part of the dog causing the cage to swing up and down freely. By means of this knife edge support an astonishing degree of sensitivity had been secured which permitted us to record even such minor movements of the animal as a change in the position of the head. The movements of the cage were graphically recorded on a slowly rotating kymograph. This was accomplished by means of a pneumograph attached to the cage in such a way as to be stretched or contracted as the cage swung up and down. This pneumograph communicated the movements of the cage through a sensitive tambour, provided with the usual recording mechanism, to the smoked surface of a kymograph drum. The kymograph records of the animal's behavior in the course of an experiment were used in conjunction with the analytical results in arriving at an interpretation. Beside this objective control and basis for estimating the degree of activity of the dogs, in a large number of the experiments another criterion has also been employed which furnished most important information in connection with the results of this metabolic study. A stethoscope placed directly over the animal's heart and fastened in that position with adhesive plaster, was so arranged as to permit the transmission of the sound of the heart beat to an observer outside the respiration apparatus. The number of heart beats per minute has been recorded by an assistant. The average pulse rate for each experimental period has been gotten from a large number of such records.

The apparatus used in these experiments has been repeatedly described in the literature and would need no further comment here. It may not be amiss, however, to make a brief statement of the way in which the temperature has been regulated. The apparatus, as is well known, is made of two galvanized iron boxes, one set within the other, with a large air space between them filled with water. The airtight closure of the apparatus is effected by means of a galvanized iron cover whose flanges dip one or two inches below the surface of the water in the jacket, when the cover is placed in position. Since this jacket contained a fairly large amount of water it was possible to regulate the temperature of the entire apparatus

with considerable ease by warming or cooling (ice) the water to the desired temperature.

The carbon dioxide was absorbed in large amounts of soda lime and determined quantitatively by weight. The oxygen consumption was likewise measured by the difference in weight of an oxygen cylinder from which the gas was supplied as it was needed. The dog was usually in the respiration apparatus for about 2½ hours at one time. A preliminary period was run until temperature conditions, etc., of the apparatus became stationary, when three or four 30-minute experiments were made, during which not only the carbon dioxide eliminated and oxygen consumed were determined but also a graphic record of the animal's behavior and extensive records of the pulse rate were obtained. This separation of an experiment into several periods of briefer duration, without interrupting the continuity of the experiment, makes it possible to eliminate parts of the experiment during which the animal has not been perfectly still. The weight of the animal was invariably determined before the experiment and the rectal temperature was measured at the beginning and immediately at the closure of the experiment. The general procedure has been to perform on the same day two consecutive experiments varying about 10° in temperature, one in the forenoon and the other in the afternoon.

First series: These experiments were made on a short haired female dog. Although this and the next series were only preliminary studies and no record of the heart beat was made, nevertheless it seems worth while recording them here. Four experiments were made with this dog at 16.2°, 16.3°, 29.6° and 31.6°C. (average temperature for the entire experiment). The results of the two experiments at the higher temperatures show a very close agreement with each other. This, however, is not the case in the other two experiments at the lower temperature, although the temperature difference between them was entirely negligible. In the experiment at 16.3°C. the metabolism was about 65 per cent greater than at 31.6°C. but the animal had been shivering during this experiment. It is interesting to point out that although in the experiment at 16.2°C. the graphic record showed the animal to be quiet, the metabolism was still 36 per cent higher than at 29.6°. The dog was tested at these temperatures on the same day. We shall see later that cold may affect the organism causing reflex responses which need not necessarily express themselves either in the form of large or even very small muscular movements (shivering). A higher degree of metabolism may be associated with a higher degree of muscular tension. The peripheral stimulation of the cold may cause reflexly a heightened state of muscle tension as well as a generally increased functional tone of the organism.

Dog 1

EXTERNAL TEMPERATURE (AVERAGE)	WEIGHT	RESPIRATORY EXCHANGE PER HOUR		R.Q.	METABOLISM PER 24 HOURS PER KILOGRAM WEIGHT
		CO_2	O_2		
°C.	*kgm.*	*liters*	*liters*		*calories*
16.2	7.57	3.04	4.09	0.74	61.3
16.3	7.37	3.51	4.63	0.76	71.8
29.6	7.33	2.04	2.84	0.72	43.7
31.6	7.37	2.06	2.84	0.73	43.6

Second series: The subject for these experiments was a long-haired male dog. These experiments are particularly interesting because they demonstrate the difficulty in giving the proper interpretation to results of respiration experiments without the aid of some objective criterion of the degree of the animal's activity. This dog was relatively restless in the course of the experiment at 11.4°, 27.4° and 28.2°, and the metabolisms calculated from the results of these experiments show little variation although they were performed at temperatures 16 to 17° apart. The experiment at 26.9°C. was successful, the graphic records showing that the dog was very quiet throughout the entire course of the experiment. In the experiment at 10.8°C. the dog was very restless and during the $2\frac{1}{2}$ hours of the entire experiment only a brief period of 30 minutes was secured during which the animal remained more or less quiet. In the table which follows the results of this experiment are given on the basis of the entire experimental period as well as on the basis of this single more or less quiet period. Even on the basis of this single period the metabolic rate was 23 per cent greater than at 26.9°. In the three separate experiments at the low temperatures (10.8 to 11.4°C.) with an average difference of only 0.6°C. between them we observe a difference in total metabolism of about 28 per cent, which is determined by the degree of restlessness with which the dog responded to the influence of the cold on each occasion.

Dog 2

EXTERNAL TEMPERATURE (AVERAGE)	WEIGHT	RESPIRATORY EXCHANGE PER HOUR		R.Q	METABOLISM PER 24 HOURS PER KILOGRAM WEIGHT
		CO_2	O_2		
°C.	*kgm.*	*liters*	*liters*		*calories*
10.8	7.38	3.87	4.99	0.78	77.6
		3.58*	4.36	0.82	68.4
11.0	7.25	2.92	4.15	0.70	64.3
11.4	7.23	2.79	3.91	0.71	60.9
26.9	7.32	2.79	3.67	0.76	57.2
27.4	7.48	3.16	3.97	0.80	61.1
28.2	7.17	2.91	3.85	0.76	61.2

* Single quiet period.

Third series: The subject for this experiment was a short-haired male dog. Only four experiments were performed with this animal, the results of which are recorded in the table below.

Dog 3

EXTERNAL TEMPERATURE	WEIGHT	RECTAL TEMPERATURE		RESPIRATORY EXCHANGE PER HOUR		R.Q.	PULSE PER MINUTE	METABOLISM PER 24 HOURS AND PER KILOGRAM
		Beginning	End	CO_2	O_2			
°C.	*kgm.*	°C.	°C.	*liters*	*liters*			*calories*
17.8	8.00	38.4	38.5	3.72	5.08	0.73	93.9	71.8
				3.36*	4.40	0.76	91.4	62.7
18.2	8.02	39.0	38.7	3.43	4.37	0.79	129.1	62.6
27.1	7.88	38.5	38.9	2.63	3.62	0.73	74.2	52.0
29.6	8.02	38.7	38.2	2.77	3.96	0.70	85.1	55.5

* Single quiet period.

We see from this experiment that there is a distinct variation in the rate of the heart beat according to the outside temperature, much higher rates obtaining at the lower temperatures. The experiment at 27.1° was the best of the entire series in the sense that the animal was quietest during that test, as was shown by the graphic record as well as by the record of the average pulse rate. The experiment at 17.8° is given on the basis of both the entire duration and also of a single 30-minute period during which the animal stayed quiet. There is a variation of about 15 per cent between the two. During the experiment at 29.6° the animal was again somewhat restless which was shown both by the graphic record as well as by the greater frequency of the heart beat. Incidentally the metabolic rate was also higher. Arranging the results in the order of the magnitude of the frequency of the heart beat, we obtain the following series:

PULSE RATE	EXTERNAL TEMPERATURE	CALORIES
	°C.	
74.2	27.1	52.0
85.1	29.6	55.5
91.4	17.8	62.7
129.1	18.2	62.6

Fourth series: The largest number of experiments under a wide range of temperature variations was performed with a female Irish terrier. Furthermore, the effect of environmental temperature was studied on the dog in its normal condition and under a condition of increased sensitivity to cold which was brought about by clipping the hair. In the first table are recorded the results of experiments extending over a period of five weeks with this animal unclipped. Experiments during which the animal was very restless are entirely omitted.

Dog 4 (A)

EXTERNAL TEMPERATURE	WEIGHT	RECTAL TEMPERATURE		RESPIRATORY EXCHANGE PER HOUR		R.Q.	PULSE PER MINUTE	METABOLISM PER 24 HOURS AND PER KILOGRAM
		Beginning	End	CO_2	O_2			
°C.	*kgm.*	°C.	°C.	*liters*	*liters*			*calories*
13.5	13.05	39.1	38.1	4.31	6.24	0.70	63.8	53.8
13.6	14.00	38.2	38.1	4.27	6.14	0.70	*52.9*	*49.3*
14.6	14.65	38.5	38.5	5.58	7.72	0.72	75.8	59.5
14.9	13.55	39.1	38.5	4.95	6.32	0.78	62.9	53.5
15.1	14.50	38.7	38.2	4.54	5.92	0.77	*68.0*	*46.5*
15.6	13.26	39.0	38.7	4.68	6.38	0.73	76.4	54.4
18.6	15.56	38.9	38.9	5.98	8.03	0.74	73.6	58.7
18.8	15.68	39.0	38.3	6.20	8.84	0.70	70.2	63.4
23.2	14.99	39.1	39.8	5.40	7.72	0.70	?*	61.5
23.4	14.00	38.6	39.0	4.28	6.16	0.70	*57.0*	*49.5*
24.4	14.50	38.7	38.7	5.34	7.47	0.71	?*	58.0
24.5	14.25	38.9	38.9	5.15	7.15	0.72	69.5	55.3

* Stethoscope off during experiment.

An examination of the data presented in this table leaves little room for doubt that the evidence is not in favor of the view that low temperature as such causes a higher metabolic rate. In the course of this long series of experiments with this dog two tests, performed on the same day, at 13.6°C. and 23.4°C. were the most perfect from the point of view of complete control of extraneous factors. It will be noted, however, that with a temperature difference of 10 degrees there has been practically no variation in the total metabolism which was 49.3 and 49.5 calories per 24 hours and per kilogram, respectively. Although these two experiments are selected for special comment from a series of about twenty experiments with the same subject, it is nevertheless true that one perfect experiment commends more confidence and has greater significance than many experiments which are not properly controlled. It will be noted that in these two experiments, the graphic records of which have shown that the dog has been remarkably quiet throughout the entire duration, the experimental findings are likewise corroborated in an objective manner by the study of the pulse rate. *It is significant, therefore, that the metabolic rate may remain unchanged within a temperature range of 10 degrees, provided complete muscular relaxation can be secured.*

The experiments which were performed on the same dog after its hair had been clipped are likewise very instructive and bear out the interpretation suggested in this paper. Of course, the clipping of the hair by diminishing the insulating capacity of the fur has made the animal much more sensitive towards cold. It is interesting to point out that following the clipping of the hair the dog, although it was fed as liberally as before, has been continually losing weight, so that at the end of three weeks it had sustained a loss of 15 per cent in weight. The first experiment under the

new conditions was performed two days after the dog was clipped, this period having been allowed for the dog to get used to the effect of the clipping. It was, of course impossible now to carry out experiments with this dog at temperatures at which before clipping fairly good results could still be obtained. Of the nine experiments performed during three weeks only those at the higher temperatures really represent a close approach to the basal metabolism. At temperatures below 20°C. the animal was usually very restless, and it was difficult to secure even single quiet periods. The increased sensitivity to cold can be shown best by comparing results of two experiments performed at nearly the same temperature. Before clipping at 10.1°C. the metabolism of the dog was 58.7 calories per kilogram and per 24 hours. The experiment was not recorded in the preceding table because the animal was somewhat restless in the course of it. It is worth while, however, to compare the results of this experiment with those obtained at 10.5°C. after the animal had been clipped. In this last experiment the metabolism was 111.9 calories. This increase of about 100 per cent is entirely due to a difference in reflex muscular response under the conditions of the two experiments; the pulse rate was likewise 25 to 30 per cent higher.

Dog 4 (B)

EXTERNAL TEMPERATURE (AVERAGE)	WEIGHT	RECTAL TEMPERATURE		RESPIRATORY EXCHANGE PER HOUR		R.Q.	PULSE PER MINUTE	METABOLISM PER 24 HOURS AND PER KILOGRAM
		Beginning	End	CO_2	O_2			
°C.	kgm.	°C.	°C.	liters	liters			calories
10.5	14.86	38.6	38.9	10.54	14.77	0.71	94.9	111.9
13.6	15.53	39.1	39.5	10.84	15.07	0.72	100.0	109.6
18.6	13.94	39.6	38.6	7.56	10.29	0.74	90.6	83.0
19.2	14.56	39.0	38.7	7.64	10.55	0.72	101.8	81.8
23.8	15.43	39.0	38.8	5.42	7.77	0.70	79.5	56.6
26.5	14.29	38.6	38.7	5.28	7.44	0.71	75.7	58.6
27.3	13.81	39.4	39.2	5.58	7.60	0.73	85.5	62.3
29.5	13.42	39.2	39.1	5.36	7.46	0.72	85.0	62.8

Arranging the results of these experiments according to the pulse rate we obtain the following series:

PULSE RATE	EXTERNAL TEMPERATURE	CALORIES
	°C.	
75.7	26.5	58.6
79.5	23.8	56.6
85.0	29.5	62.8
85.5	27.3	62.3
90.6	18.6	83.0
94.9	10.5	111.9
100.0	13.6	109.6

In a general way it may be said that, under the increased sensitivity of the dog toward cold resulting from clipping, at temperatures below 20°C., the frequency of the pulse was very great either because the animal was actually shivering or because of the heightened muscular tone resulting from the stimulation of the skin now poorly protected from the cold. The metabolism was likewise greatly increased. Under this new condition even a small variation in the environmental temperature was sufficient to bring about a large difference in the total metabolism of the animal. This increased responsiveness becomes very striking in view of the fact that previous to the reduction of the insulating capacity of the fur due to clipping this dog could bear a change of 10 degrees in temperature without any metabolic alteration. It is also obvious from the last table that as the temperature is increased above a certain optimum point the metabolism is likewise somewhat raised. This increase is due to a restlessness on the part of the experimental subject, which, though it may be small, affects sufficiently the functional state of the dog to be evinced also in a higher pulse rate.

The last series of experiments brings to light some other interesting points. It is obvious that by altering the insulating capacity of the fur by clipping the dog a very striking change has been produced in its metabolic activity. The minimum metabolism before clipping may be assumed to be 48.4 calories per kgm. and per 24 hours (average from 3 experiments), while after clipping this has risen to 57.6 calories (average from 2 experiments). This represents an increase of metabolism of about 20 per cent. It is important to note furthermore that in the former condition the minimum has been attained at temperatures of 13.6 to 15.1°C. whereas in the latter condition a much higher temperature was required (23.8 to 26.5°C.). It is well recognized that complete muscular relaxation and a resting state of digestive organs are essential in basal metabolism determinations. But it is likewise important to secure conditions under which the thermoregulatory processes of the organism are not overtaxed. This can only be realized when the heat production and dissipation are properly balanced. For correct basal metabolism results all three sets of conditions must be controlled during experiments.

Our experiments with the dog before and after clipping represent primarily a study of an acute alteration in the thermoregulatory process. Our lack of exact knowledge of the mechanism whereby this alteration is effected in no way deprives the conclusion of its validity. In these experiments we witness not only a marked change in the basal metabolism but a striking shift as well in the environmental temperature to a higher level (about 10°C.), at which the minimum metabolism occurs. Obviously, this fact has a direct bearing upon the basal metabolism technique, and in future experiments the same careful provision should be made for

securing an optimum temperature and for controlling the insulating capacity of clothing, for instance, as has already come to be regarded as indispensable from the point of view of regulating muscular and digestive activity in metabolism experiments.

We may regard that following the clipping of the hair the condition of our dog was no longer normal. Its higher metabolic activity is undoubtedly the symptom of a physiological disturbance. Even in a state of relaxation when a minimum metabolism was found, the pulse rate was considerably greater than during the period preceding the clipping. It is quite evident that it would be futile to attempt a comparison of the metabolic activity under the *same environmental temperature* with the animal clipped and unclipped, even though it were possible to secure complete muscular rest in both instances. What holds true for these experiments with our dog undoubtedly applies also to experiments where one is testing individuals whose metabolism, for pathological reasons, is greater or less than normal. In a much more limited sense, of course, this would also apply to individual variations in metabolic activity. It is necessary, therefore, to recognize that thermoregulatory equilibrium is an essential factor in basal metabolism studies, which should always be made under an environmental temperature which is best suited to the individual.

SUMMARY AND CONCLUSIONS

Surveying the results of this study as a whole it may be said that changes in temperature do not produce an alteration of metabolic activity of the tissue cells except in so far as either the low or the high temperature, through peripheral stimulation, may cause restlessness on the part of the subject or induce a state of heightened muscular tone, often without any other gross manifestations. The latter condition in an animal at rest is apparently associated with an increased tonicity of the vegetative organs as well, which finds expression, for instance, in the changes of cardiac activity. Experimental studies of the effect of temperature on the metabolism of an organism in which this matter (either of actual control of the animal's behavior or of some objective means of determining the general tone of the organism under the special experimental conditions) has been neglected, are entirely valueless from the point of view of the theoretical problem of whether or not chemical regulation of body temperature is a reality. The experimental results presented in this paper, including a study of the carbon dioxide production and oxygen consumption under various temperatures aided by a graphic study of the general behavior of the animals and the cardiac response under these different conditions, make it clear that there is no special mechanism of chemical regulation of metabolism. The metabolic changes produced under different temperatures are either the result of shivering and restlessness or, in the

absence of such gross evidences of lack of relaxation on the part of the subject, they are due to differences in muscular tonicity produced reflexly through cutaneous stimulation.[1]

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[1] Since this paper went to press I learned of Prof. W. B. Cannon's brilliant researches on the rôle of the adrenals in the control of body temperature. In a preliminary report of these experiments, recently received by me, Cannon and Querido express the view that the calorigenic reaction is in reality a double mechanism: one, a crude adjustment, depending upon an increase in metabolism resulting from such muscular responses as shivering; the other, a fine adjustment, depending upon alterations in combustion through the effect of adrenalin. The latter mechanism is thought to be identical with that postulated in Rubner's hypothesis as the chemical temperature regulation. Accepting the experimental evidence presented by Cannon and Querido without reservation, it seems nevertheless that these can be reconciled with the view developed in this paper which regards the hypothesis of a chemical regulatory mechanism as unnecessary. Space imposes a limitation of the argument, but it may be sufficient to point out here that cold acts as a peripheral stimulus both for the reflex stimulation of the sympathetic and of the secretory activity of the adrenals, which again causes a mobilization of the stores of readily combustible carbohydrates and an increased tone of the tissues. In the last analysis, therefore, the fine adjustment of the calorigenic function would still depend on the physical response of the organism, an increased muscle tonicity rather than crude muscle movements determining a higher metabolic rate, as is actually assumed in this paper.

METABOLISM OF CATTLE DURING STANDING AND LYING

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A consideration of importance in respiration calorimetry is the position of the experimental subject, as to standing and lying, because of its effect on the heat outgo and therefore on the productive utilization of nutriment.

All motion on the part of the experimental subject is significant in this relation, but in standardizing the experimental day it is practicable to consider, among the various physical activities, only those having to do with the position of the animal as to standing or lying, all others being assumed, by the method of work, to be practically alike in like intervals of time.

In the earlier work of this Institute this factor was considered, in the light of the information then at hand, in a manner fully recognized as but provisional. On account of the accumulation of much further evidence, since the adoption of our earlier method of handling this factor, it is now possible to subject the problem to more critical study and to derive a more accurate method of recognizing the facts in the computation of results.

Passing over the earlier work with various kinds of animals which has established the fact that position or posture does affect the heat production, we shall cite in introductory discussion only some of the later work, chiefly the respiration calorimetric studies of Benedict on man, to provide a background against which to view the work with cattle at this Institute.

The effect, on metabolism, of position, activity, sleep and food. From the work of Benedict and Murschhauser (1, pp. 71, 72, 74) it is seen that there is a difference in heat and CO_2 production, and O_2 consumption even with such a slight difference in posture as between standing relaxed and standing with hands resting on a support, both when the subject is fasting, and when receiving food. Thus in the two positions during fasting the heat produced per minute was 1.25 and 1.26 Calories, the CO_2 was 214 cc. and 221 cc. and the O_2 was 258 cc. and 260 cc. When the subject received light meals

[1] Acknowledgment: The authors take this opportunity to express their appreciation to Dr. E. B. Forbes for his interest and valuable assistance in the editing of this paper.

the heat was 1.42 and 1.54 Calories, the CO_2 240 and 272 cc. and the O_2 295 and 315 cc. With heavy meals the heat production was 1.56 and 1.65 Calories, the CO_2 266 and 294 cc. and the O_2 322 and 337 cc.

The data referred to also show that the heat and CO_2 production differ according to whether the meal consists chiefly of protein, carbohydrates or fat; the carbohydrate meals giving the highest heat and CO_2 production per minute.

Experiments by Benedict and Carpenter (2, pp. 242, 244) have also demonstrated that there is a difference in metabolism according to whether the subject is lying asleep, lying awake, standing or sitting. The average increase in metabolism lying awake, as compared with that lying asleep, expressed in per cent of heat and CO_2 production, and O_2 consumption, was for the heat 11.4 per cent, the CO_2 10.3 per cent, and the O_2 4.7 per cent. Similarly the increase for standing as compared with sitting was 16.5 per cent for the heat, 12.4 per cent for CO_2, and 15.2 per cent for the O_2.

These results were obtained from experiments with 6 individuals in the first case—lying awake and lying asleep—and 5 in the second; and although the individual results were not closely concordant, these average figures show a decided increase in metabolism due to differences in muscular tonus and movements.

From a large number of experiments on many different individuals the average output of carbon dioxide and heat, and the intake of oxygen, under different conditions of activity, have been computed by Benedict and Carpenter (2). By taking the values for man at rest, awake, sitting up, as 100 per cent, the values for man at rest, sleeping, would be 73 per cent heat, 70 per cent CO_2, and 79 per cent O_2. For man at rest, standing, the values would be 117 per cent heat, 112 per cent CO_2, and 116 per cent O_2. When the subject was performing very severe muscular exercise the heat was 673 per cent, the CO_2 746 per cent, and the O_2 786 per cent. For the sake of comparison the values for man at very severe muscular work have also been referred to. While in the experiments there were very marked individual differences as to minor muscular activities, the average figures given above indicate general differences in metabolism due to position and activity.

The effects of food intake on the metabolism of two subjects while in different resting positions are reported by Benedict and Murschhauser (1, pp. 63, 64, 66, 67). The average results show that in fasting the difference in metabolism between *lying* and *standing* for the one subject was a little greater than the difference between *lying* and *sitting* for the other. When food was received there was a noticeable increase in the metabolism—indicated by the increase in heat and CO_2 production and oxygen consumption—whether the subject was standing or sitting.

The after-effect of muscular work on metabolism. Benedict and Cathcart (3, p. 164) set forth the heat production per hour during sleep (1 a.m. to 7 a.m.) following different conditions of activity in experiments conducted by Benedict and Carpenter on several subjects. Thus during sleep following rest five subjects produced 69.3, 60.4, 77.2, 69.8 and 78.3 Calories per hour respectively. The first two subjects, after moderate work, produced 74.8 and 65.3 Calories per hour during sleep, and after severe work the last three of the five subjects produced 83.1, 83.3 and 83.7 Calories per hour during sleep. After very severe work the fifth subject produced 97.9 Calories per hour during sleep.

In the experiments yielding these data all work ceased at least 7 hours before the metabolism measurements began, except in the case of the very severe work, in which work ceased but 1 hour before.

In other experiments Benedict and Carpenter found that during resting periods beginning 7 to 8 hours after the cessation of muscular work the metabolism was still about 13 per cent above the normal resting value.

This disproves the idea which some entertain that after hard work—fatigue, as from long standing—the decrease in metabolism during the following resting period should be relatively greater than after very light work.

Benedict and Carpenter also present extensive experimental data on the metabolism of subjects lying on a couch, without food, before and after work (3, pp. 166–170). These data show the increase in CO_2 elimination, O_2 absorption, and heat production (computed), during periods of observation beginning from a few minutes to several hours after the cessation of work, as compared with data covering the previous period of rest.

These results show persistent increases in O_2 consumption and heat production during the resting period after work, as compared with previous resting metabolism; the CO_2 increase, however, did not show the same regularity. Of the 154 tests cited, 19 per cent showed a decrease in CO_2 production, whereas the O_2 consumption showed but one, and the heat production but two negative results. Much irregularity was also noticed, especially in CO_2 production.

Fasting experiments. From the 31 day fasting experiment on man, conducted by Benedict (4), have come some facts of significance in this discussion. A study of this fasting experiment (4, pp. 396, 397) brings out the fact that there was a considerable difference in the average metabolism of the first and the last half of the experiment. This difference may be ascribed to reduction in body weight, and to reduction of internal muscular activity during the latter half of the fast. Differences in metabolism due to position were also noticed. The average CO_2 production and O_2 consumption per hour during lying (morning, evening and night) for the first 16 days was 8.88 liters CO_2 and 11.92 liters O_2, and for the

last 15 days 7.29 liters CO_2 and 10.21 liters O_2. The data also show that metabolism during lying, at night, was less than during lying in the morning or evening.

Comparing the sitting-active and sitting-resting positions we find for the sitting-active an average of 12.02 liters CO_2 and 16.64 liters O_2 per hour for the first 16 days, and 9.73 liters CO_2 and 13.69 liters O_2 for the last 15 days. For the periods of sitting-resting the values were 9.36 liters CO_2 and 12.81 liters O_2 during the 16 days, and 7.50 liters CO_2 and 10.62 liters O_2 per hour during the last 15 days. During the standing position the values were 10.03 liters CO_2 and 13.26 liters O_2 during the first half, and 8.16 liters CO_2 and 11.28 liters O_2 per hour during the latter half of the fast.

A difference in the total daily metabolism due to position of the subject can be observed between the 21st and 22nd days of the fast. On the 22nd day the subject was lying 109 minutes more than on the 21st day, and the differences between the data of the gaseous exchange of the two days were 7.48 liters CO_2 and 8.93 liters O_2, which can be ascribed to 109 minutes difference in time spent in lying, as compared with sitting, standing and walking.

The body material katabolized per day during the first 16 days of the fast was 18.3 grams carbohydrates, 124.9 grams fat and 59.76 grams protein; and during the last 15 days, no carbohydrates, 110.1 grams fat, and 47.1 grams protein.

During the first days of the fast the oxidation of carbohydrates decreased rapidly from 69 grams on the first day to 38.5 on the third, and to nothing on the fourteenth.

The average daily heat and CO_2 production, and heat per gram of CO_2 produced during the first 16 days and the last 15 days of the fast were as follows: first 16 days—1508.6 Calories, 467.65 grams CO_2, and 3.226 Calories per gram CO_2; last 15 days—1258.2 Calories, 381.03 grams CO_2, and 3.302 Calories per gram CO_2.

From the above cited studies have been assembled the following data representing the ratios of CO_2 to heat produced in various positions, with and without food.

	grams CO_2	*Calories*
Man standing, relaxed, without food	1	2.955
Man standing, relaxed, hands on staff, without food	1	2.836
Man standing, relaxed, with food, protein-rich diet	1	3.044
Man standing, relaxed, with food, carbohydrate-rich diet	1	2.955
Man standing, relaxed, with food, fat-rich diet	1	3.269
Man sitting, without food	1	2.980
Man sitting, after light meal	1	3.004
Man sitting, after heavy meal	1	2.978
Man sitting, resting	1	2.939
Man at very severe work	1	2.633
Man during first 16 days of a fast	1	3.226
Man during 17th to 31st day of a fast	1	3.302

From these ratios of CO_2 to heat produced we learn that when the subject remains in the same position the partaking of food, whether a light or a heavy meal, causes no change in the ratio, but that a one-sided diet consisting chiefly of protein, of carbohydrates, or of fat, will produce a change in the ratio even though the subjects remain in the same position. Further, we see that severe work reduced the ratio, that fasting increased it, but that simple change in position had no definite effect upon it. With these results we shall compare similar data derived from experiments with cattle.

Metabolism in cattle affected by standing and lying. The following discussion is based on the assumption of the essential correctness of the heat measurements and analytical data as related to the periods of time represented; and the methods of computation used in the preliminary consideration are the same as were used in the earlier work of this Institute.

The results as observed, and as computed in this earlier work, however, are subjected, in the course of this paper, to a critical consideration leading to new conceptions, and to corrections in methods and final figures. It is necessary, therefore, to bear in mind the fact that the following data where designated as "provisional" do not accurately represent our final conceptions.

In the first calorimetric experiment with cattle at the Pennsylvania Institute of Animal Nutrition decided difference was observed in the heat emission during standing and lying. The animal—a steer—was quiet and regular in its habits, and usually remained for hours at a time in the same position.

The average amount of heat emitted per minute, by radiation and conduction (5, pp. 36 and 37), during the four experiments ranged from 4.135 Calories per minute with the lowest ration to 5.128 Calories with the highest ration when the animal was lying, and from 5.304 to 6.673 Calories while standing, and the ratio of heat lying to standing ranged from 1:1.283 to 1:1.349, with an average of 1:1.313.

The observation of this difference in heat emission, as measured, raised the question of method of comparison of data representing periods comprising different proportionate durations of time spent by the subject in the standing and the lying positions.

Subsequent calorimeter experiments with the same and with other steers, using feeds varying both as to kind and quantity, have revealed differences in heat emission during standing and lying similar to but usually much greater than as reported in the above (provisional) figures. This is illustrated by the data in table 1. The subject was the same as used in the work reported above but the time was a year later, and the ration a different one (red clover hay and maize meal), (6, p. 24).

In this experiment the difference in heat emission as measured during standing and lying reached a maximum of 64 per cent in one period.

In a later series of experiments (7, p. 37) an attempt was made to arrive at a basis for comparison of data from experimental periods covering different proportionate intervals of lying and standing by computing the results to an average day of 7 hours lying and 17 hours standing. This correction, obviously, would affect the figure for total heat production and, therefore, that for gain by the body.

After several years of further calorimetric experiments with steers it was decided to adopt for comparative purposes a day of 12 hours standing and 12 hours lying; and all of the subsequently published results have been computed to this basis.

In the mean time more data bearing on the subject were obtained, and a more thorough study (9), (10) was made of the problem of the influence of standing and lying on the metabolism of cattle. No change was advocated, however, in the method in use for computing results to a standard day.

TABLE 1

Heat emission by radiation, per minute of standing and lying (provisional)

PERIOD	LYING	STANDING	TIME OF STANDING	RATIO OF HEAT LYING TO HEAT STANDING
	Calories	*Calories*	*per cent*	
1	4.607	7.160	61.35	1:1.554
2	3.986	5.881	62.99	1:1.476
3	4.002	6.375	56.32	1:1.593
4	4.841	7.963	76.49	1:1.645

In the course of continued work in this field it has been increasingly manifest that the method followed in computing results to a standard day involved an error of considerable magnitude, and that the subject must be reëxamined in the light of such additional evidence as has come to hand.

As a basis for discussion we have selected the results obtained with steers A and B during years 1905, 1906 and 1907 (8).

In order to eliminate at least the greater part of the effect of the sudden irregularities in heat emission occurring immediately after a change in posture,[2] the first 10 to 20 minutes after such disturbances, as well as certain other irregular intervals were omitted in the computation of the heat emitted by radiation and conduction per minute of standing and lying as reported in the publication cited. To facilitate the study of the results of the separate standing and lying periods the data have been assembled and computed as indicated:

[2] For a description of the method employed in heat measurement in the respiration calorimeter experiments see (5, p. 24), (6, p. 20), (7, p. 19), (11, p. 1043).

1. The average heat emission per minute during standing and lying, taking the values for the different experimental periods as previously reported (8);

2. The individual intervals of standing and lying separated into those longer and those shorter than 1½ hours;

3. The difference in the heat per minute of standing and lying during the six hours following feeding;

4. The heat per minute during standing intervals just before feeding time, and in short intervals immediately after feeding.

As in the earlier work the heat emission in the experiments of 1905, 1906 and 1907 differed greatly between times of standing and lying.

Table 2 states the percentage by which the heat emission of standing is greater than that of lying (8, p. 42).

These average figures show considerable irregularity. During the experiments of 1906 and 1907 there was a greater percentage difference with increased feed, but results of the work of 1905 did not vary in this way.

Thus far then it appeared that the average increases in heat per minute during standing as compared with lying, for the whole experimental periods, ranged as in table 3. It should be borne in mind, however, that these data are merely provisional and that they are subject to revision in the light of final findings.

Similar differences in heat emission during standing and lying have been found with all the experimental animals, so that the general situation is established beyond question, though, as has been suggested, there is need for critical study of the basis for computation of the amount of this difference.

The per cent by which the recorded heat emission per minute in intervals of standing shorter than 1½ hours exceeded that of longer intervals is reported in table 4, and similar data applying to intervals of lying comprise table 5.

The time of the day and the greatly varying length of the standing intervals make this comparison somewhat unsatisfactory, but it is clear that there was recorded a greater proportionate amount of heat in the shorter than in the longer intervals.

Contributing to this different rate of heat emission there may have been a carrying over into the time beyond the excluded initial 10-to-20-minute intervals (1) of the effect of the exertion of rising, and (2) of the heat stored in the platform beneath the animal while in the lying position.

The lying intervals are even less suitable for such a comparison than are the standing intervals, but the differences between the shorter and longer intervals were computed, and the averge differences are stated in table 5.

TABLE 2

Percentage by which heat emission of standing was recorded as greater than that of lying (provisional)

STEER	PERIOD	1905	1906	1907
		per cent	*per cent*	*per cent*
A	I	50.0	34.2	31.3
A	II	37.9	44.4	48.5
A	III	48.3	32.9	34.6
A	IV	36.3	41.6	37.2
B	I	31.8	38.5	39.7
B	II	36.0	44.4	50.1
B	III	43.6	40.1	36.6
B	IV	36.9	42.0	46.4

TABLE 3

Percentage by which heat emission of standing was recorded as greater than that of lying (provisional)

STEER	YEAR	PER CENT
I	First	28.3–34.9
I	Second	47.6–64.5
A	First	36.3–50.0
A	Second	32.9–44.4
A	Third	31.3–48.5
B	First	31.8–43.6
B	Second	38.5–44.4
B	Third	36.6–50.1

TABLE 4

Per cent by which the heat emission per minute in short intervals of standing exceeded the same in longer intervals (provisional)

STEER	1905	1906	1907
	per cent	*per cent*	*per cent*
A	4.62	7.26	0.76
B	−0.55	4.62	8.0

TABLE 5

Per cent by which the apparent heat emission per minute in short intervals of lying exceeded the same in longer intervals

STEER	1905	1906	1907
	per cent	*per cent*	*per cent*
A	10.77	−1.36	1.91
B	−9.46	3.18	3.31

The foregoing results from the lying interval show much irregularity and do not point with certainty one way or the other.

We shall now consider the possibility of difference due to the time of day; and for this purpose we make use of the *longer* intervals of standing and lying, and compare those of the 6 hours after feeding with the 6 hours before feeding—the feeding-times being 6 a.m. and 6 p.m. The results of such computations for the longer standing intervals coming within the 6 hours before and after feeding are found in table 6. In a few instances an interval of standing or lying was used which overran the six-hour period.

TABLE 6

Heat emission by radiation per minute of standing within the 6 hours after as compared with the 6 hours before feeding (provisional)

Before feeding = 100 per cent

	1905	1906	1907
	per cent	*per cent*	*per cent*
Steer A after feeding	94.23	93.34	100.16
Steer B after feeding	103.20	94.60	97.02

TABLE 7

Heat emission by radiation per minute of lying, within the 6 hours after as compared with the 6 hours before feeding (provisional)

Before feeding = 100 per cent

	1905	1906	1907
	per cent	*per cent*	*per cent*
Steer A after feeding	95.99	93.31	
Steer B after feeding	100.19	100.53	

Long intervals of lying immediately preceding and immediately following feeding were few in the experiments under consideration. Such data, however, as were available have been collected, the results for the two years being given in table 7.

The figures in these two tables indicate that the heat elimination tends rather to be less after feeding than before, but not without exception.

This is contrary to what one would naturally expect, and in order to test this point a little further, a comparison was made of the standing intervals immediately before and after feeding, with results as in table 8.

Table 8 shows unmistakably that the heat elimination is less after feeding than before. We know, however, that there must be an extra expenditure of energy during eating, because of the increased muscular activity of the animal. This apparent contradiction is reconciled by

the facts that the heat production due to muscular activity during the intake of feed and water was in each of the above experiments less in amount than that required to bring the ingested feed and water up to the body temperature.

We shall next consider the relation of the apparent difference in heat elimination during standing and lying to the live weight of the animal.

Table 9 exhibits the experimental periods of each animal and year. The live weights are averages of seven weighings at the same time of day. Opposite each live weight is placed the corresponding average difference in Calories per minute between standing and lying.

Considering the heat emission as a measure of the energy metabolism there should be larger differences in heat emission between standing and lying with heavy animals than with light ones. The provisional data in table 9, however, show no such correlation, and in connection with many other observations which it is unnecessary to enumerate here have placed seriously in question the accuracy of the method previously used for correcting for the influence of position in computing to the standard day.

TABLE 8

Heat emission by radiation per minute of standing, after feeding as compared with that before feeding (provisional)

Before feeding = 100 per cent

	1905	1906	1907
	per cent	*per cent*	*per cent*
Steer A after feeding	96.91	93.34	97.39
Steer B after feeding	95.28	96.46	96.22

In order to trace the suspected error to its source the data secured in two unpublished fasting experiments of nine days' duration, the last two days of which were spent in the calorimeter, have been subjected to special consideration, since during fast the irregularities of heat emission due to feed intake and to the presence of food in the digestive tract are eliminated. In these experiments then the difference in heat emission during standing and lying should represent the muscular energy required for the support of the body, and other activities incident to the maintenance of the animal in the standing position.

In addition to the usual gravimetric method involving continuous sampling, carbon dioxide was determined gasometrically, in samples taken every half-hour, with a Sondén apparatus. During the last 24 hours of each calorimeter period oxygen also was determined on samples collected at half-hour intervals. While intermittent sampling is, in general, less reliable than continuous sampling, in these experiments the carbon dioxide excretion as determined by the two methods agreed closely.

However, in apportioning the carbon dioxide among the several intervals of standing and lying the carbon dioxide as determined gravimetrically for each 12-hour period has been split up in proportion to the amounts found for the separate intervals by the gasometric method.

A similar scheme of computation has been used to apportion the heat emitted as latent heat of water vapor among the several standing and lying intervals, the water vapor excreted by the animals during the

TABLE 9

Average live weights and difference in heat emission (radiation and conduction) per minute of standing and lying (provisional)

ANIMAL	YEAR	PERIOD	LIVE WEIGHTS	DIFFERENCE PER MINUTE STANDING AND LYING
			kgm.	*Calories*
A	1905	I	269.2	1.275
A	1905	III	271.6	1.169
A	1905	IV	276.8	1.073
A	1905	II	278.3	1.171
B	1905	IV	190.0	0.959
B	1905	III	193.1	0.991
B	1905	II	195.9	1.119
B	1905	I	199.4	0.837
A	1906	I	398.7	1.461
A	1906	III	399.4	1.149
A	1906	IV	405.5	1.534
A	1906	II	419.2	2.322
B	1906	III	295.7	1.232
B	1906	I	298.1	1.497
B	1906	IV	306.3	1.476
B	1906	II	310.5	1.897
A	1907	I	498.0	1.471
A	1907	III	508.7	1.244
A	1907	IV	512.0	1.570
A	1907	II	516.0	2.548
B	1907	I	368.5	1.701
B	1907	III	373.8	1.263
B	1907	IV	383.4	1.778
B	1907	II	383.5	2.207

12-hour period being split up in proportion to the quantities indicated by the absolute humidity of the air current during the periods of standing and lying.

The carbon dioxide-heat ratio has been computed from the respiration calorimeter tests for each interval of standing and lying during the 48 hours for cow 874, and for the last 36 hours for cow 887, the data covering the first 12 hours with this cow being considered faulty. The results of these computations are found in table 10.

TABLE 10

The ratio of the carbon dioxide elimination to the heat emission of fasting cows (Expt. 221F)

COW NUMBER	SUBPERIOD	TIME			HEAT EMITTED		TOTAL HEAT CORRECTED	TOTAL CO_2	RATIO OF CO_2 TO HEAT
		Beginning	End	Minutes	Radiated	With H_2O vapor			
					Calories	*Calories*	*Calories*	*grams*	
874	Standing 1	6:00	6:59	59.0	261.46	80.53	341.99	105.22	1:3.250
	Standing 1	7:30	7:43.5	13.5	74.44	17.35	91.79	20.41	1:4.497
	Standing 1	8:27.2	9:14	46.8	229.20	58.02	287.22	79.32	1:3.621
	Standing 1	9:58	10:12	14.0	81.79	14.87	96.66	19.77	1:4.889
	Standing 1	10:58	11:11	13.0	69.46	13.12	82.58	17.62	1:4.687
	Standing 1	11:54	1:09.8	75.8	352.13	75.69	427.82	107.82	1:3.967
	Standing 1	1:25.8	2:47	81.2	357.00	78.31	435.31	117.74	1:3.697
	Standing 1	2:59.5	4:20.5	81.0	356.49	74.86	431.35	118.59	1:3.637
	Standing 1	5:44	6:00	16.0	101.84	21.47	123.31	23.23	1:5.308
Total				400.3			2318.03	609.72	1:3.802
874	Lying 1	6:59	7:30	31.0	83.37	39.83	123.20	46.84	1:2.630
	Lying 1	7:43.5	8:27.2	43.7	127.03	54.14	181.17	67.27	1:2.693
	Lying 1	9:14	9:58	44.0	111.40	49.30	160.70	70.35	1:2.284
	Lying 1	10:12	10:58	46.0	174.30	47.65	221.95	66.13	1:3.356
	Lying 1	11:11	11:54	43.0	160.33	43.85	204.18	62.58	1:3.263
	Lying 1	1:09.8	1:25.8	16.0	53.26	15.61	68.87	22.29	1:3.090
	Lying 1	2:47	2:59.5	12.5	33.43	11.80	45.23	17.95	1:2.518
	Lying 1	4:20.5	5:44	83.5	235.72	76.27	311.99	108.07	1:2.887
Total				319.7			1317.29	461.48	1:2.854
874	Standing 2	6:00	7:36	96.0	359.08	98.47	458.31	139.83	1:3.278
	Standing 2	8:40.5	9:28.5	48.0	192.08	45.14	237.76	69.86	1:3.403
	Standing 2	10:14.5	12:05.5	111.0	423.34	134.03	558.62	157.33	1:3.551
	Standing 2	1:29.5	6:00	270.5	1059.62	284.15	1346.83	422.23	1:3.190
Total				525.5			2601.52	789.25	1:3.296
874	Lying 2	7:36	8:40.5	64.5	168.67	57.10	226.50	82.32	1:2.751
	Lying 2	9:28.5	10:14.5	46.0	120.42	40.26	161.20	63.64	1:2.533
	Lying 2	12:05.5	1:29.5	84.0	216.86	70.57	288.38	108.01	1:2.670
Total				194.5			676.08	253.97	1:2.662
874	Standing 3	6:00	7:12	72.0	301.05	98.39	398.62	134.21	1:2.970
	Standing 3	8:17.5	9:28.5	70.75	300.86	76.88	377.74	102.84	1:3.673
	Standing 3	11:27	11:52.5	25.50	137.34	24.97	162.31	35.18	1:4.614
	Standing 3	1:30	2:01.5	31.50	154.21	42.45	196.66	46.42	1:4.236
	Standing 3	3:40.25	3:52.5	12.25	69.26	10.17	79.43	19.05	1:4.169
	Standing 3	5:02.5	6:00	57.50	267.73	57.21	324.94	86.81	1:3.743
Total				269.5			1539.70	424.51	1:3.627

TABLE 10—*Concluded*

COW NUMBER	SUBPERIOD	TIME			HEAT EMITTED		TOTAL HEAT CORRECTED	TOTAL CO_2	RATIO OF CO_2 TO HEAT
		Beginning	End	Minutes	Radiated	With H_2O vapor			
					Calories	*Calories*	*Calories*	*grams*	
874	Lying 3	7:12	8:17.5	65.5	199.11	81.25	280.36	94.59	1:2.964
	Lying 3	9:28.25	11:27	118.75	336.90	118.58	455.48	164.61	1:2.767
	Lying 3	11:52.5	1:30	97.5	293.50	106.14	399.64	133.03	1:3.003
	Lying 3	2:01.5	3:40.25	98.75	276.86	91.97	368.83	133.61	1:2.760
	Lying 3	3:52.5	5:02.5	70.0	213.67	63.86	277.53	92.17	1:3.011
Total				450.5			1781.84	618.0	1:2.883
874	Standing 4	6:00	6:25.2	25.2	112.03	28.96	140.20	42.75	1:3.279
	Standing 4	7:26	10:12.25	166.25	702.27	181.54	883.81	236.89	1:3.731
	Standing 4	12:03	12:25.5	22.5	128.32	23.02	151.34	36.36	1:4.162
	Standing 4	2:30	6:00	210.0	857.48	199.04	1056.52	308.07	1:3.429
Total				423.95			2231.87	624.07	1:3.576
874	Lying 1	6:25.2	7:26	60.8	171.20	62.61	233.81	82.78	1:2.824
	Lying 1	10:12.25	12:03	110.75	310.43	108.35	418.78	151.27	1:2.768
	Lying 1	12:25.5	2:30	124.5	370.28	115.89	486.17	167.95	1:2.895
Total				296.05			1138.76	402.00	1:2.833
887	Standing 2	6:00	2:11.8	491.8	1668.16	403.99	2069.46	615.43	1:3.363
	Standing 2	3:00	6:00	180.0	659.10	157.71	815.82	262.77	1:3.105
Total				671.8			2885.28	878.20	1:3.285
887	Lying 2	2:11.8	3:00	48.2	133.39	38.32	171.45	72.99	1:2.349
887	Standing 3	6:00	11:07	307.0	1106.58	237.71	1342.48	398.13	1:3.372
	Standing 3	11:46	6:00	374.0	1380.87	275.89	1656.76	496.83	1:3.335
Total				681.0			2999.24	894.96	1:3.351
887	Lying 3	11:07	11:46	39.0	110.42	29.05	139.47	59.19	1:2.356
887	Standing 4	6:00	6:00	720.0	2462.26	607.57	3076.23	948.43	1:3.243

Cow 874 changed position frequently, and great differences are observed in the carbon dioxide-heat ratio for the several intervals, the average for standing being 1:3.551 and for lying 1:2.831. The ratio for standing is apparently theoretically impossible, since the value for pure animal fat is 1:3.387.

With cow 887, which stood practically all the time, the carbon dioxide-heat ratio for standing was 1:3.292, the short standing intervals giving higher ratios of CO_2 to heat than the longer intervals. These data cast

doubt on the accuracy of the division of the heat or the CO_2, or both, between short periods of standing and lying.

The data of table 10 computed to values per minute comprise table 11.

With cow 874 the percentages by which the outgo of carbon dioxide and heat during standing exceeded the values for lying were 9.81 and 37.71, respectively.

With cow 887 the CO_2 outgo per minute during short lying periods was higher than the average for standing, but less than the value found for standing at the time of change of position. These high values for CO_2 during short lying intervals lose significance, however, in consideration of the fact that most of the variations in bodily activity occurring during the day are included in the longer standing intervals.

The total heat per minute, per 100 kgm. live weight, was 1.181 Calories for cow 874, and 1.426 Calories for cow 887; that is, the smaller cow gave

TABLE 11

Carbon dioxide and heat per minute during standing and lying, and during entire fasting periods (provisional)

	FASTING LIVE WEIGHT	PERIOD	RATIO OF CO_2 TO HEAT	PER MINUTE	
				CO_2	Heat
	kgm.			*grams*	*Calories*
874	400	Standing	1:3.551	1.5115	5.3674
		Lying	1:2.831	1.3765	3.8977
		Total	1:3.176	1.4875	4.7240
887	301	Standing	1:3.292	1.3130	4.3230
		Lying	1:2.352	1.5158	3.5656
		Total	1:3.249	1.3212	4.2924

off 21 per cent more heat per kgm. live weight than the heavier cow. This inequality may be ascribed to differences in individuality, in body surface, and in time spent in the standing position.

During the last day in each of the two fasting experiments samples of the ventilating air current were taken each half hour, and the CO_2 and O_2 determined gasometrically. The percentages of CO_2 and O_2 thus obtained, by volume, are set forth in table 12.

With cow 887, which stood almost the entire time, the percentages by volume of CO_2 and O_2 were remarkably uniform. With cow 874, which changed position frequently, we find more change in the percentages of these gases, but it will be noticed that with the decrease in CO_2 there is an increase in the O_2 percentage. This is an indication of a change in the intensity of metabolism. In no instance is there any change in these gases which would correspond to, or begin to account for, the difference in ratio of CO_2 to heat which has been observed at this Institute during standing as compared with lying.

Further, in the case of a cow in milk, on full feed, the CO_2 and O_2 determinations made during the hours immediately preceding feeding

TABLE 12

Variations in the composition of the air current from the respiration calorimeter during the fasting experiments

TIME	CO_2	O_2	TIME	CO_2	O_2	TIME	CO_2	O_2
Cow 874								
	per cent	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*
6:30 p.m.	0.220	20.666	2:30 a.m.	0.172	20.754	11:30 a.m.	0.165	20.742
7:00	0.220	20.668	3:00	0.165	20.738	12:00	0.162	20.740
7:30	0.189	20.709	3:30	0.167	20.764	12:30 p.m.	0.197	20.695
8:00	0.167	20.746	4:00	0.189	20.685	1:00	0.170	20.730
8:30	0.183	20.724	4:30	0.167	20.733	1:30	0.154	20.733
9:00	0.177	20.729	5:00	0.161	20.750	2:00	0.172	20.729
9:30	0.196	20.698	5:30	0.178	20.739	2:30	0.166	20.733
10:00	0.174	20.749	6:00	0.204	20.720	3:00	0.179	20.719
10:30	0.164	20.751	6:30	0.201	20.708	3:30	0.174	20.728
11:00	0.168	20.745	7:00	0.169	20.611	4:00	0.170	20.729
11:30	0.171	20.762	8:30	0.180	20.714	4:30	0.191	20.695
12:00	0.188	20.714	9:00	0.171	20.731	5:00	0.183	20.706
12:30 a.m.	0.167	20.736	9:30	0.172	20.719	5:30	0.181	20.705
1:00	0.161	20.753	10:00	0.198	20.688	6:00	0.211	20.661
1:30	0.163	20.758	10:30	0.188	20.677			
2:00	0.181	20.729	11:00	0.168	20.725			
Cow 887								
6:00 p.m.	0.162	20.733	2:30 a.m.	0.162	20.748	10:30 a.m.	0.152	20.741
6:30	0.164	20.734	3:00	0.158	20.738	11:00	0.154	20.747
7:00	0.157	20.752	3:30	0.158	20.733	11:30	0.152	20.760
7:30	0.164	20.797	4:00	0.154	20.736	12:00	0.159	20.744
8:00	0.164	20.744	4:30	0.156	20.752	12:30 p.m.	0.160	20.746
9:00	0.154	20.744	5:00	0.154	20.740	1:00	0.160	20.745
9:30	0.158	20.755	5:30	0.160	20.747	1:30	0.157	20.740
10:00	0.156	20.752	6:00	0.154	20.749	2:00	0.176	20.722
10:30	0.160	20.751	6:30	0.156	20.750	2:30	0.164	20.728
11:00	0.162	20.742	7:00	0.172	20.717	3:00	0.165	20.736
11:30	0.182	20.727	7:30	0.163	20.747	3:30	0.172	20.727
12:00	0.175	20.729	8:00	0.160	20.749	4:00	0.175	20.725
12:30 a.m.	0.165	20.730	8:30	0.170	20.734	4:30	0.164	20.727
1:00	0.172	20.727	9:00	0.168	20.718	5:00	0.169	20.733
1:30	0.166	20.745	9:30	0.162	20.750	5:30	0.155	20.740
2:00	0.168	20.728	10:00	0.160	20.739	6:00	0.175	20.733

showed no indications of a difference in the character of metabolism during standing and lying. Thus, for example, with cow 874, during a lying period the air samples contained 0.313 per cent CO_2 and 20.625

per cent O_2; and, during the next following standing period, 0.349 per cent CO_2 and 20.583 per cent O_2, which is an increase of 0.036 per cent CO_2 and a decrease of 0.042 per cent O_2. Observations on other animals, which received feed, yielded data of like significance.

The foregoing observations on the metabolism of man and of cattle, in relation especially to change of position, lead to the following summary statement:

With man a change in position causes a change in the rate of metabolism; change in position alone causes little or no change in the CO_2-heat ratio; ingestion of food increases the metabolism; a one-sided diet causes a change in the CO_2-heat ratio; severe work causes a decrease of the CO_2-heat ratio; the after-effect of work is noticeable during several hours in an increased metabolism as compared with the previous period of rest; and the CO_2-heat ratio is increased by fasting.

With cattle the observed experimental data (provisional) show apparently unreasonably large differences in the heat emission during standing as compared with lying, showing no correlation between these differences and the live weights of the animals.

The CO_2-heat ratio during standing *appeared* to be greater than during lying.

During fasting the CO_2-heat ratio approaches that of fat.

During fasting the observed CO_2-heat ratio for standing was a theoretically impossible value, in the case of one cow (874).

A great variation was noted in the CO_2-heat ratio of different intervals of standing, or lying, during the same day.

During fasting the respiratory quotient does not follow the CO_2-heat ratio as found for intervals of standing and lying, by the provisional method thus far discussed.

The foregoing discussion of the increase of heat production during standing as compared with lying points definitely to a storage of heat during the time the animal is lying, and the subsequent radiation of this stored heat during the succeeding intervals of standing.

That such a storage of heat was possible, in the platform on which the animal reclines in the calorimeter, was early recognized in connection with the experimental technic of this Institute; but that the magnitude of this storage was sufficient seriously to affect the determinations of heat emission has not been recognized. Furthermore, until the fasting experiments to which reference is made in the preceeding pages, were concluded, no data were available for computing the true increase of heat production for standing as compared with lying.

Assuming that the CO_2-heat ratio is the same during standing as during lying, it is possible by means of the CO_2-heat ratio, to determine the magnitude of this instrumental error, and to compute a correction for the same.

Determination of the instrumental error, by means of the observed differences in CO_2 and heat production of standing and lying. We have observed, in the course of our experiments, that the CO_2 production varies during the course of the day on account of irregularity in the excretion of the gases of fermentation. During a single day, therefore, a disproportionate amount of the fermentation-CO_2 may be credited to either the intervals of standing or of lying. Hence, in order to obtain a reasonably reliable average, it is necessary to make use of the data from a considerable number of experiments.

The separation of the daily CO_2 and heat production to correspond to intervals of standing and of lying presents numerous difficulties, and not all experiments are suitable as bases for the computation of the instrumental error. Among the many difficulties encountered in such division of the CO_2 and heat are uncertainty of heat measurement during very short periods, due to the lag of the apparatus and the over-lapping of observations, and irregularity in the excretion of the carbon dioxide of fermentation. Also unduly short periods of standing or lying, and frequent changes in position, make the determination of residual CO_2 in the calorimeter chamber difficult and uncertain; and, further, the division of the heat emitted into the several intervals of standing and lying, especially in the case of the shorter intervals, is affected by a calorimeter capacity correction which theoretically should be a variable, but for which a fixed value must be used.

There are other irregularities, in the daily life of the animal, over which the investigator has no control; for example, the difference between the heat emission of standing and lying is affected by the amount and temperature of the drinking water, and by the time elapsed since drinking; by the amount, temperature and kind of feed consumed, especially by its protein content, and by the lapse of time since eating; and by the losses of heat in excreta and in milk; and by the time and positions in which these losses occur. It is also impracticable accurately to apportion the latent heat of water vapor to correspond with the position of the animal, hence the entire heat emission cannot be consistently divided according to intervals of standing and lying.

It must be clearly understood that an absolutely correct numerical value for the amount of heat stored in the material upon which the animal lies cannot be obtained from the data at hand. However it is possible to compute a correction of high percentage validity which can be used to good purpose until structural changes can be made in the apparatus such as will obviate the necessity of such a procedure.

A storing of heat in the materials underneath the body of the animal while lying, and the radiation of this heat again during standing, signifies that a certain amount of heat should be subtracted from that measured during standing and added to that observed during lying.

On the assumption that the ratio of carbon dioxide to heat production remains the same throughout any given experiment, it is possible to compute the amount of heat which must be subtracted from the standing and added to the lying intervals to equalize this ratio.

A number of experiments furnishing suitable data for this computation have been selected. The corrections have been computed and recorded in table 13. These experiments comprise sixteen calorimeter periods—seven 48-hour experiments with a steer receiving various amounts of alfalfa hay alone, or alfalfa hay and corn starch; seven 24-hour experiments with 3 cows on full feed, five being milking periods and two dry periods; also one 48-hour experiment with a fasting cow; and one 24-hour experiment with a second fasting cow.

In making the application of such a computation the correction must apply to single intervals of standing, and not to the day or to the experiment, as a unit; hence the computed value for heat emission during the aggregate of intervals of standing in a given calorimeter period was divided by the number of times the animal arose and stood for an interval of 25 or more minutes. Intervals of standing of less than 25 minutes duration were included as proportionate parts of 25-minute intervals. The determination of 25 minutes as the minimum interval of standing to which the full correction should apply was made in consideration of the mass and material of the platform, and the area exposed to the ventilating current.

As will later appear this correction was finally adopted only for certain specific purposes, and not for use in computing to the standard day, since we later arrived at an understanding of the problem in the light of which it is no longer necessary or desirable to separate the heat emission corresponding to intervals of standing and lying.

The specific purposes referred to are *a*, the correction of the total heat production, in case of the animal being in different position as to standing or lying at the beginning and the end of the calorimetric period, and *b*, the derivation of a correction covering the continuing increase of heat emission during standing as compared with lying.

It will be noted that the correction of the daily heat emission of standing and lying to conform to a constant CO_2-heat ratio is of considerable magnitude. The average as found in these 16 experiments was 43.45 Calories for each time of rising.

This value is an instrumental correction for the calorimeter of this Institute. It not only corrects for the storage of heat in the platform on which the animal lies but also includes other minor factors.

The data for steer J, period I, and cow 874, period III, are of especial interest in this connection on account of the very different planes of nutrition represented, the corrections, however, being about the same.

TABLE 13

The correction of the heat emission of standing and lying to conform to a constant CO_2-heat ratio

EXPERIMENT	ANIMAL	PERIOD	POSITION	MINUTES	TOTAL CO_2	OBSERVED HEAT	CORRECTION	HEAT CORRECTED	CO_2-HEAT RATIO		STANDING PERIODS	
									Before correction	After correction	Number	Correction per interval
					grams	*Calories*	*Calories*	*Calories*				*Calories*
216	Steer J	I	Standing	1055.2	5654.36	13250.12	−807.15	12442.97	1:2.343	1:2.201	19.7	40.97
		I	Lying	1824.8	8404.65	17688.11	+807.15	18495.26	2.104	2.201		
		II	Standing	1110.8	3221.64	8700.04	−829.24	7871.40	2.701	2.443	19.0	43.64
		II	Lying	1769.2	4611.35	10437.62	+829.24	11266.86	2.261	2.443		
		III	Standing	908.4	3222.82	7822.21	−429.17	7393.04	2.427	2.294	14.0	30.65
		III	Lying	1971.6	6234.50	13872.56	+429.17	14301.73	2.225	2.294		
		IV	Standing	1139.9	2672.62	7482.20	−714.60	6767.60	2.811	2.532	14.0	51.00
		IV	Lying	1740.1	3645.00	8515.25	+714.60	9229.85	2.336	2.532		
		V	Standing	1076.5	4595.96	10944.53	−343.86	10600.67	2.381	2.306	22.6	15.21
		V	Lying	1803.5	6481.99	14606.98	+343.86	14950.84	2.253	2.306		
		VI	Standing	855.5	2874.81	7568.41	−725.91	6842.50	2.636	2.380	15.6	46.53
		VI	Lying	2024.5	6316.45	14308.22	+725.91	15034.13	2.265	2.380		
		VII	Standing	1018.5	2661.63	7211.68	−476.76	6734.92	2.709	2.530	16.1	29.51
		VII	Lying	1861.5	3843.07	9247.64	+476.76	9724.40	2.406	2.530		
221A	Cow 631	I	Standing	458.0	1712.68	4925.76	−867.25	4058.51	2.876	2.370	12.2	71.09
		I	Lying	982.0	3286.80	6921.43	+867.25	7788.68	2.106	2.370		
		II	Standing	534.1	2088.38	5365.62	−704.92	4660.70	2.569	2.232	15.0	46.99
		II	Lying	905.9	3379.02	5836.18	+704.92	7541.10	2.023	2.232		
		III	Standing	515.3	2228.72	5719.21	−658.19	5061.02	2.566	2.271	11.2	58.77
		III	Lying	924.7	3685.47	7710.84	+658.19	8369.03	2.092	2.271		
	615	II	Standing	909.9	3351.10	8467.57	−783.24	7689.44	2.528	2.295	13.0	60.25
		II	Lying	530.1	1964.45	3729.51	+783.24	4507.64	1.896	2.295		
		III	Standing	897.1	3854.88	8686.39	−204.66	8496.38	2.257	2.204	8.2	24.96
		III	Lying	542.9	1767.05	3704.67	+204.66	3894.68	2.088	2.204		
	579	I	Standing	994.6	4319.94	10234.22	−238.52	9995.70	2.369	2.314	7.5	31.80
		I	Lying	445.4	1581.59	3421.05	+238.52	3659.57	2.163	2.314		
		II	Standing	809.6	3429.07	8340.68	−555.69	7784.99	2.432	2.270	13.3	41.78
		II	Lying	630.4	2406.38	4907.50	+555.69	5463.19	2.039	2.270		
221F	874	III	Standing	1619.25	2447.55	8691.12	−730.55	7960.57	3.551	3.252	17.2	42.47
		III	Lying	1260.75	1735.46	4913.97	+730.55	5644.52	2.831	3.252		
	887	III	Standing	1352.8	1773.16	5884.51	−118.89	5765.62	3.319	3.252	2.0	59.44
		III	Lying	87.2	132.18	310.92	+118.89	429.81	2.352	3.252		
Average												43.45

Steer J was on full feed, and gave off 30,038 Calories in 48 hours, was standing during 19.7 intervals (according to the method of computation), and exhibited an apparent difference of 11.36 per cent in the daily CO_2-heat ratios of standing and lying.

Cow 874 was fasting, and gave off in 48 hours 13,605 Calories. She was standing during 17.2 intervals, and exhibited an apparent difference of 25.43 per cent in the daily CO_2-heat ratios of standing and lying.

The correction in the former case was 40.97 Calories, and in the latter 42.47 Calories.

Corrected increase of heat emission of standing as compared with lying, with fasting animals. In order to derive the true increase of heat emission due to standing, for use in computing the heat emission to a standard day (in relation to standing and lying), the heat emission of a fasting animal during standing and lying, corrected to conform to a constant CO_2-heat ratio, was used.

Thus cow 874 gave off 4.9162 Calories per minute while standing, and 4.4771 Calories per minute while lying. Computed to a standard day of 12 hours' standing and 12 hours' lying, the true increase of heat of standing as compared with lying is 316.15 Calories.

In other words, an animal weighing 400 kgm. gives off, while standing, 26.34 Calories per hour more than while lying, on account of the accompanying and resultant differences in rate of metabolism.

In the derivation of this figure for true increase of heat emission of standing as compared with lying the corresponding instrumental correction per time of rising was 42.47 Calories. If, however, the average correction of 43.45 Calories per time of rising is applied in correcting the heat emission in this particular case the increase (extra energy per hour of standing) is 24.92 Calories.

The fasting animal has been selected for this particular computation because the data are not complicated by the influence of the presence of large quantities of feed in the digestive tract.

Method of computing the heat production of the standard day. Before entering upon a discussion of the method of correcting the heat production to a standard day of 12 hours' standing and 12 hours' lying we submit the following definitions of certain terms used.

Maintenance requirement: The maintenance requirement of an animal, for energy, is that amount of feed energy necessary to keep the animal in energy balance under normal conditions of life, above the critical temperature.

In discussions of results of the work of this Institute the maintenance requirement is that amount of energy necessary to keep the animal in energy balance above the critical temperature, during a standard day of 12 hours standing and 12 hours in the lying position.

Heat increment: In relation to the utilization of the feed the heat increment, in general terms, is the energy expenditure of feed utilization. More specifically it is that part of the metabolizable energy which can not serve for either maintenance or production. It is, therefore, the energy loss, or expense, incident to realizing the net useful effect of the feed. The heat increment is often called the work of digestion.

Heat production: The total heat production is the sum of the maintenance quota of net energy and the heat increment, or work of digestion (which includes the heat of bacterial fermentation).

It is assumed that a day's heat increment, or work of digestion, is not a tered by differences in time spent standing and lying.

In the light of this assumption the increase of heat production of standing as compared with lying is an increase of the requirement for maintenance.

The significance and the consequences of this assumption may be illustrated by consideration in the form of a problem involving an experimental day in which the animal stood for 9 hours and lay down for 15 hours.

(1) Heat produced in 15 hours' lying = work of digestion (d) + net energy for maintenance (n)

(2) Heat produced in 15 hours' lying = work of digestion (d_1) + net energy for maintenance (n_1)

On a 12 hour basis:

(3) Heat produced in 12 hours' standing = work of digestion (d_2) + net energy for maintenance (n_2)

(4) Heat produced in 12 hours' lying = work of digestion (d_3) + net energy for maintenance (n_3)

Adding (1) and (2):

(5) Total heat produced in 24 hours = $(d + d_1) + (n + n_1)$

Adding (3) and (4):

(6) Total heat produced in 24 hours on the basis of a standard day = $(d_2 + d_3) + (n_2 + n_3)$

The difference between (5) and (6) = $(d_2 + d_3) + (n_2 + n_3) - [(d + d_1) + (n + n_1)]$

But $d_2 + d_3 = d + d_1$

Therefore

The difference between (5) and (6) = $(n_2 + n_3) - (n + n_1)$ = the difference in the maintenance requirement of net energy due to the addition of 3 hours of standing and the subtraction of 3 hours' lying.

The increase of heat emission during standing as compared with lying, for an animal weighing 400 kgm., was found to be 26.34 Calories per hour.

Then $(n_2 + n_3) - (n + n_1) = 3 \times 26.34$.

That is (3) + (4) = (1) + (2) + (3 × 26.34).

In the light of this explanation, therefore, it is possible to compute the total heat production for a standard day of 12 hours' standing and 12 hours' lying without altering the amount of heat due to work of digestion —which is correct.

From the foregoing explanation it is clear that it is possible logically to compute the heat production as observed to terms of a standard day without the separation of the heat to correspond to the intervals of standing and lying as they occurred in the experiment.

As to the accuracy of this figure, 26.34 Calories per hour, it must not be forgotten that it is derived from but a single experiment. From our most critical review of the data through which it has been derived, how-

TABLE 14

Factors for correction of total heat production to a standard day of 12 hours standing and 12 hours lying

LIVE WEIGHT	NET ENERGY PER HOUR
kgm.	*Calories*
275	20.5
300	21.7
325	22.9
350	24.1
375	25.2
400	26.3
425	27.4
450	28.5
475	29.5
500	30.5
525	31.5
550	32.5
575	33.5

ever, we are convinced that it is a close approximation of the true increase of heat production of standing as compared with lying. In respects which are quite beyond the control of the experimenter the particular experiment on which this factor is based was practically ideal for our purpose, and many more fasting experiments might be conducted without the recurrence of these favorable conditions. These were the unusual quietness and regularity of behavior of the cow, the fact that she stood and reclined during similar parts of the day, and that the separate intervals of standing and lying were of such length as to preclude the probability of confusion of heat of standing with heat of lying.

Since more energy is required to support a heavy animal than a light one the increase of heat production of standing as compared with lying should be expected to vary with the size of the animal. This increase,

therefore, being considered a factor of the maintenance requirement of energy, has been computed for animals of different size, according to the usual method in relation to the $\frac{2}{3}$ power of the live weight.

To apply the above correction to an experiment it is necessary to know the total heat producton per day, and the time the animal spent in the standing and lying positions. If the animal stood less than 12 hours, then the difference, in hours, between 12 and the number of hours the animal stood, is multiplied by the figure corresponding to its live weight (table 14), the product being added to the total heat production. If the animal stood more than 12 hours the correction should be subtracted from the heat production.

Thus may be eliminated one of the most time-consuming portions of the routine procedure of work in direct calorimetry as it has been done at this Institute, at the same time eliminating a serious source of error. Further, this new procedure is equally applicable in indirect calorimetry, in the balance method as well as in the respiratory quotient method.

SUMMARY

A critical study of the heat emission of cattle in the standing position as compared with lying revealed the fact that the division of the total heat emission between intervals of standing and lying, as directly observed by means of the respiration calorimeter of the Pennsylvania Institute of Animal Nutrition, contains an error of considerable magnitude. This error is due chiefly to a storage of heat in the platform upon which the animal reclines, and to the subsequent radiation of this heat when the animal rises. It tends, therefore, to make the heat emission of the standing intervals too great, and that of the lying intervals too small.

The existence of this error was revealed by *1*, an unaccountably large excess of heat in the standing as compared with the lying intervals; *2*, the fact that there is no correlation between these increases of heat and the live weight of the animal; *3*, a larger ratio of CO_2 to heat observed during standing than during lying; *4*, a great variation in the observed ratios of CO_2 to heat in different intervals of either standing or lying during the same day; *5*, the fact that the respiratory quotients during fasting do not correspond to the CO_2-heat ratios as observed for periods of standing and lying; and *6*, the fact that with one cow during fasting the average observed CO_2-heat ratio for standing was a theoretically impossible value.

On the assumption that the CO_2-heat ratio is unaltered by change of position, and that the division of the carbon dioxide between intervals of standing and lying is correct, the magnitude of this instrumental error was computed, the average of sixteen determinations being 43.45 Calories, which represents the full capacity of the apparatus to store heat each time

the animal is in the lying position, and to radiate it in the subsequent period of standing.

The true increase of heat emission due to standing was determined from an experiment with a fasting animal, after correcting the heat emission of the standing and lying intervals to conform to a constant CO_2-heat ratio. This value was found to be 26.34 Calories per hour for a cow weighing 400 kgm.

A new method has been evolved for computing the total heat production to a standard day, as to standing and lying, without first separating the heat to correspond to the intervals of standing and lying. This method is based on the following considerations:

1. The heat production of an animal is the sum of the maintenance quota of net energy and the energy expenditure due to the consumption of the feed,—commonly called the work of digestion.

2. Differences in the time spent by the animal in standing and lying during the day are assumed to influence the maintenance requirement of net energy, because of the differences in muscular activity, but are practically without effect on the work of digestion of any given ration.

3. The increase of energy expended by a fasting animal in the standing position as compared with lying is assumed to be a measure of the increased maintenance requirement of net energy due to standing. This can be computed according to the size of the animal regardless of the plane of nutrition.

The new method of computing the heat production to a standard day as to standing and lying is applicable to indirect as well as to direct calorimetric experiments, since the only data necessary are the total daily heat production, the weight of the animal, and the total time of standing.

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VISCERO-MOTOR REFLEXES. II

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Our knowledge of viscero-motor reflexes began with the conception of Mackenzie (1) that the abdominal rigidity occurring in visceral disease was reflex in origin. His idea was that inflammation of a viscus elicited, reflexly, a contraction of the abdominal muscles, which afforded protection for the underlying, irritated viscus. That this interpretation was correct was proved by Sherrington (2), who stimulated the central ends of various visceral nerves and thus evoked, reflexly, contraction of the abdominal muscles.

In a recent paper (3) it was shown that, in the viscero-motor reflexes, besides the abdominal muscles, the muscles of the hindlimbs are involved as effectors; the hindlimbs execute movements and assume postures of a defensive nature. The suggestion was advanced that the drawing-up of the legs in abdominal visceral disease in man is a protective reflex, the purpose of which is to diminish the intra-abdominal pressure, to which the inflamed or irritated viscus is subjected.

In the present research a study was made, with the aid of graphic records, of the contractions of certain of the muscles involved in these reflexes. Some observations were also made on the reflex contraction of the diaphragm.

METHODS. The decapitate cat of Sherrington (4) was employed, the decapitation being performed under profound anesthesia. Inflation of the lungs was carried on by a respiration pump.

In recording the contraction of the *rectus abdominis* the muscle of one side was detached from its attachment to the pubic tubercle and was connected by a silk thread with a crank-lever, pulling against a light, coiled spring.

When records of the limb muscles were desired the other muscles of the extremity were paralyzed by appropriate nerve section or their tendons were cut. These procedures were carried out prior to decapitation. The tendon of each muscle to be studied was connected by a silk thread to a lever of the type just mentioned, the limb itself being secured by suitable means of fixation. For recording the reflex contractions of the diaphragm

a median slip was isolated and connected, by a thread passing over a pulley, to the crank-lever; the xiphisternum was firmly clamped.

The visceral nerves to be stimulated were isolated by careful dissection after the arrangements for recording were complete. Each nerve-plexus was ligated with fine silk and the electrodes were applied just central to the ligature. The electrodes were of the shielded type, the platinum terminals being 2 mm. apart. The faradic current was employed for stimulation, two Edison storage cells furnishing the current for the primary coil of the inductorium (Stoelting); a rheostat and ammeter were included in the primary circuit, the rheostat being so adjusted that, with the interrupter in action, the ammeter recorded 0.38 ampère. At 135 mm. secondary distance the current was just detectable on the tip of the tongue. In one experiment (on the diaphragm), owing to the high degree of reflex excitability of the animal, the core was removed from the primary coil. Under these conditions the threshold current for the tongue was obtained with the secondary at 100 mm.

Stimulation was initiated by opening a key, which, at the same instant, actuated a signal writing on the drum. Adequate precautions were taken to avoid vitiation of results by escape of current.

Results. *Rectus abdominis.* Faradization of the central ends of the coeliac nerves yields, in the *rectus abdominis*, a powerful, tonic contraction, which rises to a maximum and then gradually declines, a considerable "after-discharge" (5) following the stimulation. The tonic contraction is accompanied by clonic contractions, large and small, the latter being of the nature of a fine, rapid tremor (fig. 1).

Iliopsoas. The reflex effects elicited in this muscle by faradization of the central ends of the superior mesenteric nerves consist in large and small clonic contractions, associated with some degree of tonus. The clonic contractions are larger, while the tonus is less marked than in the *rectus abdominis.* An after-discharge succeeds the stimulation (fig. 2).

Tibialis anterior and gastrocnemius. Simultaneous records were made of the contractions of these muscles. Stimulation of the central ends of the coeliac nerves yields, in the *tibialis anterior*, a series of clonic contractions, showing considerable summation, as in an incomplete tetanus; the contractions are accompanied by a tonus, which augments and then declines; a fine tremor also occurs. The gastrocnemius executes one or two brief contractions in response to stimulation of the coeliac nerves (fig. 3).

Faradization of the central ends of the superior mesenteric nerves elicits, in the *tibialis anterior*, a succession of clonic contractions, which ascend to a summit as in the case of an incomplete tetanus; associated with these contractions is a considerable amount of tonus, which first increases and then subsides suddenly. There follow a few clonic contractions with but little tonus. A fine tremor is also noticeable. The reflex response of

the *tibialis anterior* to stimulation of the superior mesenteric nerves thus resembles closely its response, just described, to stimulation of the coeliac nerves.

The effects evokable in the *gastrocnemius* by faradization of the superior mesenteric nerves consist in a couple of sharp, brief contractions, which may be succeeded by a fine tremor (fig. 4).

Fig. 1. Reflex contraction of *rectus abdominis* from coeliac nerves. Sec. dist. 140 mm. In this figure upward movement of signal indicates commencement of stimulation. Time = ½ second.

Fig. 2. Reflex contraction of *iliopsoas* from superior mesenteric nerves. Sec. dist. 130 mm. In this and succeeding tracings downward movement of signal indicates commencement of stimulation. Time = ½ second.

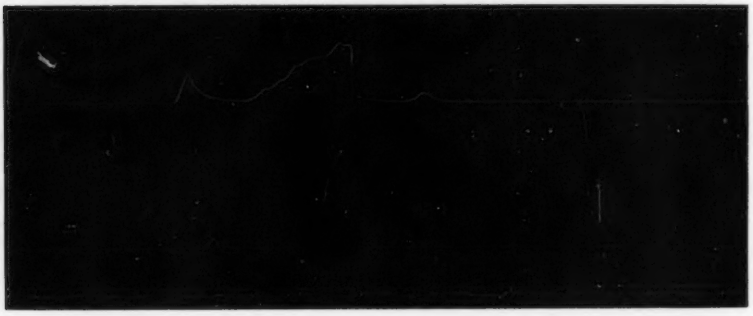

Fig. 3. Reflex contractions of *tibialis anterior* (above) and *gastrocnemius* (below) from coeliac nerves. Sec. dist. 130 mm. Time = ½ second.

By the use of corresponding arcs (fig. 4) the first contraction of the *gastrocnemius* is found to be synchronous with that of the *tibialis anterior*. A definite after-discharge is not present in the response of these muscles.

Noteworthy is the predominance of effects in the flexor muscle (tibialis anterior) over those in the extensor muscle (gastrocnemius).

Reflex effects in other muscles. Diaphragmatic viscero-motor reflex. That a large number of muscles, besides those referred to, are concerned in the

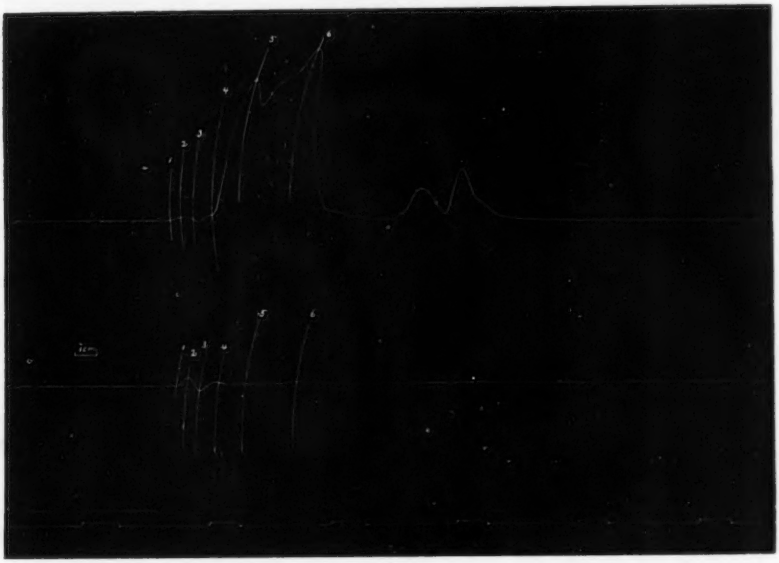

Fig. 4. Reflex contractions of *tibialis anterior* (above) and *gastrocnemius* (below) from superior mesenteric nerves. Sec. dist. 150 mm. *1*, *2*, *3*, *4*, *5*, *6*, corresponding arcs. Time = ½ second.

Fig. 5. Reflex tonus increase of diaphragm (diaphragmatic viscero-motor reflex) from coeliac nerves. Sec. dist. 75 mm. (core removed from primary). Rhythmical movements caused by respiration pump. Time = ½ second.

viscero-motor reflexes may be readily determined by inspection and palpation; the abdominal muscles, both ventral and dorsal groups respond vigorously and to these must be added the diaphragm. Faradization of the coeliac nerves elicits reflexly a tonic contraction of the diaphragm, as may be seen by reference to figure 5. The rhythmical movements are due

to the blasts of the respiration pump; the tonic contraction of the diaphragm is indicated by a rise in level of the excursions; evidences of a fine tremor are also to be noted.

DISCUSSION. Tonic spasm of the diaphragm, occurring as a clinical condition in man, is described by Eppinger (6). To this condition Pottenger (7) applies the term "diaphragmatic viscero-motor reflex" and ascribes the muscular spasm to reflex influences from inflamed thoracic or abdominal viscera.

Of the same nature as these clinical conditions is, doubtless, the reflex contraction of the diaphragm experimentally induced from the coeliac nerves, as described above; accordingly, I would apply to it the term "diaphragmatic viscero-motor reflex."

It was already stated that in the limb muscles clonic effects predominate, though tonus is also present; in the abdominal muscles tonic effects predominate, though some clonic contractions occur. We may term these two types of viscero-motor reflexes *viscero-clonic* and *viscero-tonic* respectively. In the case of the viscero-tonic reflexes activities are taking place, which greatly increase the tonus of certain skeletal muscles, particularly those of the abdominal wall.

The idea of skeletal muscular tonus prevailing at the present time was developed by Sherrington (8), (9), who showed that it is a reflex postural activity dependent on stimulation of receptors in the muscles themselves. The distribution of the tonus is precisely adapted to the posture assumed; for example, in such a posture as standing, the muscles exhibiting tonus are those which actively oppose gravity, the "antigravity" muscles (9).

It appears altogether likely, now, that the viscero-tonic reflexes contribute to or modify in some way the tonus of the body muscles, especially those of the abdominal wall, which, according to Sherrington (8), probably share in postural tone. It was shown by Simpson and myself (3) that traction on the mesentery of the stomach or intestine yields reflex contraction of the muscles of the abdominal wall. The Pacinian corpuscles present in the mesentery of the cat (10) are quite possibly the receptors concerned in this reflex. The effect of gravity, especially when acting on a well-filled stomach or intestine, will clearly be to exert a pull on the mesentery and thus elicit the reflex increase of tonus in the abdominal muscles. In the viscero-tonic reflexes of the abdominal wall we have, therefore, important factors, capable of modifying materially the postural tonus of this group of muscles. Experiments by Simpson and myself (3) show a viscero-tonic influence on the postural tonus of the limb muscles also.

Besides these reactions to gravity there are others. Simpson and I (3) found that distention of the stomach, together with the inevitable traction on its mesentery, yields reflex increase of tonus in the abdominal wall.

In this case the viscero-tonic reflex appears as a protective mechanism, which guards the abdominal viscus against over-distention and rupture.

While it is likely that this protective mechanism was developed primarily to oppose the effects on the viscera of gravity and excessive stretching there is little doubt that the same fundamental mechanism is brought into play, to an exaggerated degree, in the severe irritation of visceral disease.

SUMMARY

1. The motor responses occurring in the viscero-motor reflexes were studied graphically in a number of muscles.

2. The *rectus abdominis* exhibits a great increase of tonus with but slight clonus; an "after-discharge" occurs (viscero-tonic type of reflex).

3. The *iliopsoas* and *tibialis anterior* exhibit marked clonus with moderate increase of tonus; a slight after-discharge is present in the iliopsoas (viscero-clonic type of reflex).

4. The *gastrocnemius* executes a couple of short, sharp contractions.

5. The diaphragm shows a powerful increase of tonus ("diaphragmatic viscero-motor reflex").

6. Gravity, by acting on a well-filled stomach or intestine, exerts traction on the mesentery and thereby evokes the viscero-tonic reflex in the abdominal muscles. The "postural tonus" of these muscles is thus modified.

7. Distention of a viscus like the stomach also yields the abdominal viscero-tonic reflex, which thus appears as a protective mechanism, guarding the viscus against over-distention and rupture.

8. The same viscero-tonic reflex mechanism is brought into play in the irritation of visceral disease.

I desire to thank my assistant, Dr. R. A. Waud, for help in performing the experiments reported in this paper.

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THE CIRCULATORY RESPONSES OF MAN TO ANOXEMIA

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In the study of the physiologic changes which have been found to occur during anoxemia, including the effects of high altitudes, there has been a disposition to try to explain each of the observed changes as a compensatory reaction, which serves to increase the supply of oxygen to the tissues at a time when there is difficulty in maintaining an adequate flow of this vital gas to the needy cells. Conspicuous among the reactions of the body to a reduced oxygen supply are certain circulatory changes that may be interpreted as meaning an increase in minute-volume of blood flow. From the experience in the use of a rebreathing method of producing anoxemia at the laboratories of The School of Aviation Medicine, of the Army, both during the war and more recently, it has been the disposition of some observers to consider that the circulatory reactions constitute the only trustworthy criteria of compensatory ability. One writer (26) stated that

> In the compensation for low oxygen, the circulation appears to be the factor of first importance. Changes in respiration, in concentration or chemical constitution of the blood, or in the mechanism of gaseous exchange are important, but they are able to make good only partially for the deficiency. The factor of chief importance with a wide range of adaptability is the rate of blood flow, both in general and as regards special parts.

It is our purpose to briefly review past researches that bear on the subject of circulation during anoxemia and to present new data which are opposed to the theory that the circulatory responses constitute a means of compensation to low oxygen.

Schneider (18) has pointed out that of the several responses made to low oxygen, especially as experienced during a residence at a high altitude, those affecting the circulatory system are most temporary and tend to disappear, even though they may be maintained in part; while the respiratory and blood changes are of a permanent character. In a review of the literature on this subject he has shown that the heart usually responds to low oxygen by an acceleration in its frequency of beating and that there are individual variations, some of which are the result of differences in the physical condition of the men observed.

Much of the data that led to the belief in an increase in the volume of blood flow has been of an indirect nature. Lutz and Schneider (15) in reporting experiments on the effects of anoxemia by rebreathing and by means of a low pressure chamber stated: "Throughout our experimental work with low oxygen we have assumed that an increase in the rate of the heart beat, the arterial pressures being maintained within normal limits, meant an increase in the per-minute output of the heart". Their experiments were supposed to give evidence of increased blood flow in the increased pulse rate, in a moderate fall in the diastolic arterial blood pressure and a rise in pulse pressure that resulted not only from the fall in diastolic pressure, but also frequently from a rise in the systolic pressure.

A statistical analysis of a large number of cases of anoxemia by rebreathing made by Schneider and Truesdell (19) indicated that a large increase in the breathing is more frequently associated with the toleration of an extremely low oxygen than is a large increase in pulse rate. From that study it became clear that the increase in the minute-volume of breathing was the compensatory factor of first importance. In a special study (21) of ten groups selected from 2000 cases of various circulatory conditions such as high and low systolic pressures, high and low diastolic pressures, large and small pulse pressures, rapid and slow pulse rates, and cases of a systolic pressure rise and of a fall on standing, it was found that all responded in a similar manner and compensated to equally low percentages of oxygen. In other words, none of these circulatory conditions handicapped the body in compensating to low oxygen.

The old view that changes in the minute output of the heart were indicated by the changes in the product of pulse rate by pulse pressure has had to be given up, since Skeleton (24) clearly showed that the pulse pressure is not an index of the heart output per beat. She found that a short, sudden contraction of the heart will raise the pressure more and give a bigger pulse pressure than a slow prolonged contraction which expels the same amount of fluid.

The relative volume of blood flow during anoxemia has usually been estimated by some such indirect method of observation as the above. The Anglo-American Pike's Peak Expedition (5) used recoil board records and pulse pressure data to estimate the volume of the heart strokes, and concluded that the heart strokes were practically unchanged at the high altitude. Sisco and Schneider (20) later used the same method, and also determined the amount of blood flow through the hands with Stewart's hand calorimeters. They concluded that the output per heart stroke was unchanged in four subjects and decreased in another, while the flow of blood through the hands was increased from 30 to 76 per cent in six subjects at the high altitude.

Kuhn (13), with the respiratory method of Plesch, found the output of the heart per beat decreased in two, unchanged in one and increased in the fourth man at 11,000 feet. The minute-volume was calculated to have increased in these men between 3.2 and 28.1 per cent. Hasselbach and Lindhard (7), with three men in a pneumatic chamber by means of the nitrous oxide method of Krogh and Lindhard, failed to find evidence of an increased rate of circulation.

More recently Doi (4) determined the volume of blood passing through the lungs of an anesthetized cat during acute anoxemia by observations on the oxygen consumption and the oxygen content of the arterial and venous blood. He concluded that the minute-volume of blood flow underwent practically no change, and that the stroke volume of the heart was decreased.

The expedition to the Peruvian Andes Mountains (1) studied the problem of blood flow by means of several methods that employ Fick's principle; namely, that the amount of oxygen absorbed or carbon dioxide eliminated by the body per minute divided by the oxygen or carbon dioxide difference of the arterial and venous blood gives the minute-volume in liters. It was found that with the 3 methods used it was not possible to be certain of a change of 20 per cent in either direction; but, nevertheless, they did not show either a rise or a fall in the minute-volume when at Cerro, altitude 14,200 feet.

The experimental results presented in this paper have been collected under two conditions. In the majority of cases the anoxemia was produced by rebreathing 52 liters of air, from which the carbon dioxide was removed by a sodium hydroxide absorber. As the oxygen content decreased various circulatory observations were made at regular and frequent intervals. In the other type of experiment the subject was kept under a constant low barometric pressure in a low pressure chamber while observations were made. The topics of study include the changes of anoxemia in the capillary and venous blood pressures, in the flow of blood through the blood capillary, in the hand volume, in the rate of blood flow through the hand, and in outflow of blood per minute from the heart.

Capillary pressure and blood flow through the capillary. The capillary blood pressure was determined in 10 rebreathing experiments on 5 subjects with the Danzer-Hooker (3) micro-capillary tonometer. The mean amount of change in the pressure has been calculated for the group for each decrease of 1 per cent in the inhaled oxygen and the results are plotted in a curve that is given in figure 1. Anoxemia did not affect the capillary pressure. While variations occurred in the pressure, they ranged above and below normal; and the mean capillary pressure was not definitely changed at the time when the low oxygen effects were most pronounced on the pulse rate, and on the arterial and venous blood pressures.

The flow of blood through the capillaries was watched in the skin capillaries of the nail groove of a finger (14). The flow varied in velocity and in the condition of the blood. There were marked individual differences. The changes most frequently observed were as follows: At first the flow was swift and homogeneous; as the inhaled oxygen decreased a slight granularity appeared in the blood, this gradually increased with the reduction of oxygen; the granularity was often followed by clumping of the red corpuscles. The clumps sometimes became larger as anoxemia progressed. At this

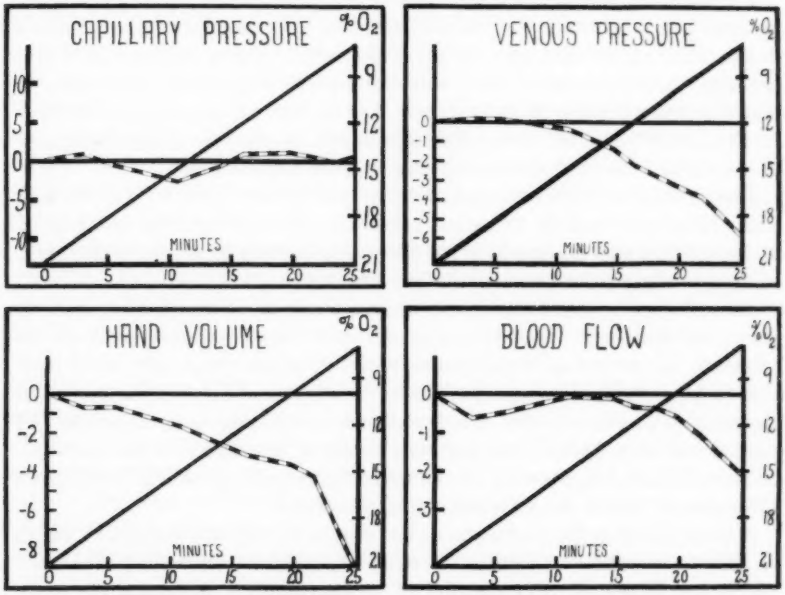

Fig. 1. Curves of means for circulatory changes during rebreathing as the oxygen decreases from 20.93 to 7 per cent.

Capillary pressure means record the changes in millimeters Hg. Venous pressure means are for the amount of change in centimeters H_2O. Hand volume changes are in cubic centimeters. Blood flow changes are in grams of blood.

stage corpuscle free cylinders of plasma often separated the clumps. Frequently the rate of flow was visibly retarded and in some instances all motion ceased, while at other times backward pulsations occurred. Even under normal conditions, variations in the rate of flow were common, and at times a slight degree of granularity was present; but a very slow flow and a lack of motion were only observed toward the end of the period of rebreathing, when anoxemia was extreme and the subject approached mental inefficiency. Not all of our subjects showed the anoxemia changes in the capillary flow, some maintained a rapid homogeneous flow throughout the

entire period of rebreathing, some experienced a slight retardation and a moderate degree of granularity; while others showed the entire series of changes outlined above. In those subjects who showed a slowing of the flow, the rate again increased when they were restored by fresh air. Sometimes in the post-anoxemia period the acceleration was immediate and at other times somewhat delayed. An attempt was made to record changes in the size of capillaries, but no instance of a well-defined change was observed.

Venous blood pressure. The influence of anoxemia on the venous blood pressure has been studied by three methods of lowering the oxygen pressure in the respired air; namely, by a reduced atmospheric pressure in a low pressure chamber, and by two methods in which a normal atmospheric pressure was maintained as the percentage of respired oxygen was lowered. In the first of these two series the oxygen was reduced by rebreathing, and in the second the atmospheric air was diluted with nitrogen.

The venous pressure was measured by the Hooker-Eyster (11) method with Hooker's venous pressure glass capsule and by the method of Y. Henderson and Haggard (8) for observing the height of the hydrostatic column in the veins of an arm of the reclining subject.

The effect of anoxemia upon the venous pressure, as has previously been shown by Schneider (22), is almost without exception a lowering of the pressure, the extent of which varies with individuals and with the reduction in the supply of oxygen. There was approximately the same fall in the venous pressure under the 3 methods of causing anoxemia; the fall ranged between 29 and 146 per cent under a low atmospheric pressure, between 32 and 134 per cent by the rebreathing method and between 22 to 115 per cent under the nitrogen dilution method.

A curve showing the mean amount of change in pressure is given in figure 1. This was prepared from a set of 15 experiments on 9 individuals for which the Hooker-Eyster method of determining the pressure was used. In order to prepare the curve the amount of variation in pressure was first tabulated for each case at each oxygen per cent from 21 to 7 and the means then calculated. The mean normal pressure for the group was 9.4 cm. H_2O, while at 18 per cent of oxygen it was 9.5 cm. A fall in the pressure first appeared at 15 per cent of oxygen in 7 experiments, the mean change was then only 0.5 cm. At 13 per cent of oxygen 10 cases showed some degree of fall; the mean change, when compared with the normal, was a drop of 1.5 cm. At 12 per cent oxygen one more case had responded, and the mean fall was 2.2 cm. At 9 per cent oxygen the mean fall was 3.9, at 8 per cent 4.7, and at 7 per cent 5.9 cm. In one experiment, in which the final oxygen was 6.6 per cent, the pressure never became subnormal. This man had a normal pressure of 14.6 cm. His pressure at first gradually rose and was 19.5 cm. at 15 per cent oxygen, it then maintained a level down to 10

per cent oxygen, after which it gradually fell to 15 cm. at 6.6 per cent. In 14 of the 15 experiments and in 8 of 9 individuals some fall in the venous pressure occurred during the period of rebreathing. The mean fall amounted to 62.8 per cent.

In 4 of these experiments the venous pressure was negative toward the close of the run. The extreme amount of change occurred in 2 individuals. In one the normal pressure was 5.8 cm. and fell to −4.4 cm. at 6.7 oxygen. In this case the venous pressure remained stationary at 0 from 13 to 8.5 per cent oxygen. At this stage the systolic and diastolic arterial pressures, which had been at normal or slightly above, began to drop and at the same time the venous pressure decreased further. The rate of the heart beat accelerated to the close of the period of rebreathing, rising from a normal of 80 beats to 105. In the second case the normal venous pressure was 7.8 cm. and fell to −2.4 cm. at the close of the rebreathing period. This subject showed, in 3 experiments, a gradual fall in the diastolic arterial pressure in the latter part of the rebreathing period, which began at about 12 per cent of oxygen.

Another, and larger, group of experiments was made in which the venous pressure was recorded by the method used by Henderson and Haggard. There were 32 experiments on 22 men during rebreathing. A curve showing the changes in pressure was prepared by the same plan that was used in our first group. The mean duration of these experiments was 25 minutes and 10 seconds, and the mean for the oxygen at the end of the experiment was 6.8 per cent. The mean normal pressure was 6.6 *cm.* The curve of change during rebreathing was much like that given in figure 1. Some fall in the venous pressure was already present at 15 per cent of oxygen, mean 1 cm. The mean fall was 2.5 cm. at 12 per cent, 3.6 cm. at 9 per cent, and 4.8 cm. at 6.8 per cent of oxygen.

The fall in pressure for the second group ranged from 1 to 11 cm. H_2O. In 5 cases the pressure was negative toward the close of the run by from 1 to 2.7 cm. In 4 cases the venous pressure was not influenced by the anoxemia. The mean pressure for the entire group at the close of the rebreathing was 1.8 cm. The mean fall in pressure was 72.7 per cent.

The effect of a gradual reduction of the atmospheric pressure in a low pressure chamber, in which the pressure was lowered at approximately the same rate as the oxygen decreased in rebreathing experiments, also caused a fall in the venous blood pressure. The onset of the change occurred in several cases near 500 mm. Hg barometric pressure. The amount of change and the level at which it began corresponded to the other two series.

Ordinarily the venous pressure came promptly back to normal when the subject was given oxygen or restored to fresh air, but in some instances the return was slow and required as much as 10 minutes for recovery.

An attempt has been made to account for the anoxemic fall in the pressure, but it has not been entirely successful. No constant relation was found between the changes in the rate of heart beat and those of venous pressure, or between the arterial blood pressure changes and the fall in venous pressure. Likewise attempts to associate these changes with those of the minute-volume, depth and rate of breathing also failed to show a causal connection. Y. Henderson, Prince and Haggard (9) obtained a striking and long-continued fall in venous pressure during and after forced breathing. Under exposure to low oxygen of our type of experiment the breathing is not visibly increased and, therefore, not forced. Schneider and Truesdell (19) found, from an analysis of a large number of cases, that from 4 to 5.5 liters in the minute-volume of breathing at 7.2 per cent oxygen is a good average increase. Among our cases there were, however, instances of but little and others of an extraordinarily large respiratory increase in which the fall in venous pressure was approximately the same. No correlation could be established between the respiratory and venous pressure changes of anoxemia. Hooker (12), by causing alterations in the intrathoracic pressure by breathing through a mask into which fresh air streamed under a positive or negative pressure, obtained responses in the venous pressure which he attributed to purely mechanical conditions. A sharp rise in venous pressure occurred when the air in the mask was under a positive pressure and a gradual fall when under a negative pressure. In our experiments the mechanical explanation was eliminated because in the nitrogen dilution method the air was introduced into the mask under a slight positive pressure, and in some of the rebreathing experiments a positive expiratory pressure was necessitated by a caking of the sodium hydroxide in the absorbing cylinder and this occurred without causing an alteration in the character of the curve of the venous pressure response to the anoxemia. Furthermore, the change in venous pressure was no greater in the low pressure chamber experiments than in the other two methods used to cause anoxemia in which the normal atmospheric pressure was maintained. Therefore, in both conditions of positive and negative pressure, the venous pressure fell as the partial pressure of oxygen decreased in the respired air.

Because of the fact that a vaso-constriction of the splanchnic vessels results in a rise in venous pressure, it was thought that by increasing the intra-abdominal pressure during the exposure to the conditions of anoxic anoxemia we might prevent the fall in venous pressure. To test this several subjects wore a tightly laced corset during rebreathing tests, with the result that the fall in venous pressure was at least lessened and delayed and in several instances even prevented. The following examples illustrate the action. In 3 experiments on V. A. S., in which numbers 1 and 2 were without and number 3 with the corset, very tightly laced, and the final

oxygen was 10.4, 11 and 9.9 per cent respectively, the following results were obtained:

	NORMAL	5 MINUTES	13 MINUTES	18 MINUTES	23 MINUTES	27 MINUTES
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
1	9.4	7.3	6.3	5.8	4.2	3.6
2	7.9	7.6	5.5	2.7	−0.7	−2.4
3	10.1	9.4	10.0	10.9	9.5	7.5

In a pair of experiments on E. C. S., no. 1 without and no. 2 with the corset, with final oxygen 7.7 and 8.3 per cent respectively, the following results were obtained:

	NORMAL	5 MINUTES	13 MINUTES	18 MINUTES	23 MINUTES	27 MINUTES
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
1	20.7	20.2	19.9	19	15.2	14.0
2	19.0	19.9	19.5	20.6	19.5	19

In 2 experiments on J. K. S., no. 1 without and no. 2 with corset, with the final oxygen respectively 7.3 and 6.6 per cent, the following were obtained:

	NORMAL	5 MINUTES	13 MINUTES	18 MINUTES	23 MINUTES	27 MINUTES
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
1	5.2	6.6	6.0	5.1	2.1	1.4
2	2.3	2.0	3.3	3.9	6.5	5.3

These are our best results obtained under an increased abdominal pressure. In all cases some fall in the venous pressure occurred during the last part of the experiment, in some of the corset experiments the pressure does not become subnormal, but after rising during the mid-period of rebreathing falls slightly without again reaching normal. Apparently, therefore, a part of the fall in the venous pressure that occurs during anoxemia is due to the accumulation of blood in the splanchnic vessels and its failure to return to the great veins in as great a volume as ordinarily.

Hand volume. The hand volume changes were determined by means of a plethysmograph in 18 experiments on 13 subjects during the progressively developing anoxemia induced by the rebreathing method. A curve showing the mean amount of change at various percentages of oxygen was prepared by tabulating the amount of change in cubic centimeters at several percentages of oxygen as compared with the hand volume prior to rebreathing, and then calculating the mean amount of change at each oxygen point selected. The curve so established is given in figure 1. At the first two

points, 19 and 18 per cent oxygen, there is no definite change in the hand volume; but by the time 15 per cent of oxygen has been reached the mean volume has already decreased 1.3 cc.; and from there on it decreases slowly as the available oxygen is reduced, until at 7 per cent oxygen the mean decrease in hand volume has reached 10.4 cc. In the period from 9 to 8 per cent oxygen the volume decreases about 2 cc. and between 8 and 7 per cent 4.3 cc. more. There were 4 experiments in which the hand volume did not decrease.

The after-effect was not as carefully studied as the changes during the development of anoxemia. However, in 5 subjects, who were carried down to 7 per cent of oxygen, the recovery was rather slow. The average volume increased only 2 cc. in 7 minutes. In another subject the hand volume decreased 10 cc. down to between 8 and 7 per cent of oxygen; and then, in the post-experiment period in 12 minutes, gradually rose to 3 cc. above normal. The time used for the restoration of normal hand volume varied greatly, in some individuals it was accomplished within 5 minutes and in others not within 15 minutes.

Blood flow in the hand. By means of Stewart's hand calorimeters (25) the rate of blood flow per 100 cc. of hand volume was determined during rebreathing experiments of approximately 25 minutes duration in which the final oxygen was ordinarily between 8 and 7 per cent. There were 29 experiments on 11 subjects. Of this number 22, or 75.9 per cent, showed a slowing of the flow during the latter part of the experiment; while in 7, or 24.1 per cent, the flow was unaffected.

In accordance with the method of visualizing the effects of anoxemia in other circulatory factors, a curve showing means for the differences in the rate of blood flow has been prepared and is given in figure 1. The normal reaction, as thus expressed, is a slight, really negligible, early slowing followed by a return to the normal rate, which is then maintained until the inhaled oxygen has decreased to 13 per cent. From then on, as the oxygen supply further decreases, the flow of blood through the hand is moderately retarded until at 7 per cent oxygen the rate of flow shows an average decrease of 2.2 cc. per minute for a 100 cc. of hand volume. The normal rate of blood flow through the hand ranged from 2 to 12.6 cc. per 100 cc. of hand volume. The mean flow was 6.7 cc. Consequently the mean retardation amounted to 32.8 per cent.

That the retardation in blood flow is a reaction to anoxemia is indicated by a study of the after-effect that occurred when the subjects were restored to normal atmospheric air. In all except 5 of the 29 experiments the rate of flow accelerated in the post-period. Sometimes the flow in this period was greater than the normal, but in most cases the return was only to or almost to normal. This return, like the recovery of hand volume, was slow rather than immediate as was to be expected. During the first 5

minutes of the post-period the mean flow per 100 cc. of hand volume increased 1.02 cc.; at the end of 15 minutes it was just about back to normal.

The minute-volume output of the heart. A knowledge of the changes that occur in the peripheral circulation during anoxemia does not give information as to the state of the circulation in the brain and other vital organs under the same condition. It is desirable, therefore, to learn what happens to the stroke volume of the heart during the various stages of anoxemia. Several methods have been suggested for the measurement of the stroke volume or, what amounts to the same thing, of the amount of blood that passes from the right to the left side of the heart per minute.

We have tried to use several of these methods with subjects under a low barometric pressure in a low pressure chamber. None of them has proved to be satisfactory under our conditions of work. The several methods require the measurement of the respiratory metabolism, which determines the oxygen intake or carbon dioxide output per minute, and the difference in the content of oxygen or carbon dioxide in the arterial and venous blood. The oxygen or carbon dioxide content of the arterial blood, it has been assumed, may be derived from the oxygen or carbon dioxide dissociation curves of the blood by applying to the proper curve the tension of either gas in the alveolar air of the lungs. The estimation of the oxygen or carbon dioxide content of the venous blood is not so easy. For this measurement we have tried the methods of Barcroft, Roughton and Shoji (2), Henderson and Prince (10), Meakins and Davies (16), and the triple extrapolation of Redfield, Bock and Meakins (17). We have found that those methods which use the carbon-dioxide content of the venous blood were the most unsatisfactory for our purpose at the low barometric pressure. This is due to the fact that anoxemia results in a lowered content of oxygen in the blood, an increase in hemoglobin and red blood corpuscles, and a reduction in the carbon dioxide content of the blood because of increased breathing. Since the effects of these conditions on the carbon dioxide dissociation are not definitely worked out, the carbon dioxide dissociation curves were considered untrustworthy for our need.

Our best results, and these are not entirely satisfactory, were obtained by the method of Barcroft, Roughton and Shoji. The basis of the determination is oxygen. In order to secure an estimation of the oxygen content of the venous blood the subject breathes nitrogen from a bag, holds his breath awhile, then exhales into a container so that the alveolar air may be sampled. The subject gets on well enough at an atmospheric pressure of 760 mm., but at a pressure of 350 mm. the nitrogen causes dizziness and sometimes a momentary loss of consciousness. Because of this many experiments conducted at 350 mm. were failures. We used pressures between 380 and 350 mm. in order to secure a well-defined anoxemic acceleration of the heart.

The results of 11 experiments on 4 subjects appear in table 1. The rate of blood flow per minute at 760 mm. ranged between 3.12 and 5.87 L., average 4.82 L.; and at 350 mm. between 2.49 and 4.54 L., average 3.45 L. The pulse rate ranged between 53 and 84, average 76, at 760 mm.; and between 77 and 99, average 86, at 350 mm. If the method used to determine the oxygen content of the venous blood is reliable then a decided reduction in the stroke volume and minute output of the heart is indicated. We are not satisfied that the determinations for 350 mm. are reliable. Even in the most satisfactory experiments the extreme oxygen want caused by inhaling the nitrogen made it difficult to give proper attention to exhaling a good sample of the alveolar air.

TABLE 1

SUBJECT	EXPERIMENT	O_2 CONSUMPTION PER MINUTE		DIFFERENCE IN O_2 BETWEEN ARTERIAL AND VENOUS BLOOD		BLOOD FLOW PER MINUTE		PULSE RATE	
		760 mm.	350 mm.	760 mm.	350 mm.	760 mm.	350 mm.	760 mm.	350 mm.
		cc.	*cc.*	*cc.*	*cc.*	*liters*	*liters*		
C. R. J.	1	246	224	5.6	5.6	4.38	4.00	62	86
	2	281	285	4.8	7.7	5.85	3.70	71	87
H. H. F.	1	283	249	5.1	9.2	5.55	2.71	78	77
	2	264	284	4.5	7.1	5.87	4.00	84	93
R. W. C.	1	211	245	4.0	5.4	5.22	4.54	80	99
	2	294	242	5.7	6.7	5.16	3.61	73	91
	3	232	229	4.4	9.2	5.27	2.49	53	77
E. C. S.	1	225	211	4.8	6.9	4.69	3.06	72	88
	2	226	201	6.1	6.7	3.70	3.00	69	84
	3	219	224	7.0	7.3	3.12	3.01	66	78
	4	242	257	5.8	6.7	4.17	3.84	69	87
Average.....		248	241	5.3	7.1	4.82	3.45	76	86

We have made determinations of the oxygen content of the venous blood of the arm, under the same barometric pressures that were used in the above experiments, which indicate, when considered with other available data, that the minute-volume of the heart is not increased in anoxemia. The venous blood data were published by Schneider, Truesdell and Clarke (23), and may be found in table 3 of their article. In 14 of 16 of those experiments the difference between the oxygen content of the arterial and venous blood of the arm averaged 6.5 cc. at 760 mm. and 5.6 cc. at 350 mm. On applying these values to the average consumption of oxygen for the same experiments, the minute volume of blood flow was apparently 4.48 L. at 760 mm. and 4.86 L. at 350 mm. This would mean

an increase of 8.5 per cent in the rate of blood flow in the arm at 350 mm. However, it should be recalled that our observations with the Stewart calorimeter on the blood flow through the hand, the study of the hand volume changes, and the microscopic inspection of the capillary blood flow indicated a reduction in the rate of peripheral blood flow. The seeming increase shown by the study of the composition of venous blood is due to the fact that, because of reduced metabolism of the arm, less oxygen is withdrawn from the blood during a sojourn at 350 mm. than when at 760 mm. The data in their entirety indicate that the metabolism of the muscles is temporarily reduced (23) in anoxemia. In the light of these observations, it is clear that the gases of the blood of the arm can not safely be used to estimate the minute volume of blood flow. Only the content of gases of the mixed blood of the right side of the heart would give satisfactory data for the determination of the minute volume output of the heart.

While we are doubtful as to the blood flow values obtained at a barometric pressure of 350 mm. with the Barcroft, Roughton and Shoji method, we do feel certain that our data, when taken in their entirety, clearly indicate that the minute volume of blood flow from the heart is not increased during anoxemia. A more exact study of the stroke volume of the heart during anoxemia will be made in this laboratory with animals.

In the light of the new results, it may be well to restate the circulatory changes published from this laboratory. It has been our custom, in the past, to regard the ordinary type of circulatory reactions as a means of compensation to an oxygen deficiency. This position must now be abandoned.

The circulatory reactions may be considered under 2 conditions—one in which anoxemia is carried to the stage of unconsciousness by the process of a continuous and gradual reduction of the oxygen in the air breathed; and the other in which the anoxemia is carried to a stage sufficient to cause certain well-defined physiological responses, after which a level is maintained in the supply of available oxygen.

Under the first condition the heart rate augments during oxygen reduction. Acceleration is slight and very gradual in the early stages of reduction; becomes more pronounced at from 15 to 12 per cent oxygen; and may be profound as the limit of endurance is approached, which may be between 7 and 6 per cent (15). The arterial blood pressure may follow 2 general courses designated as the non-fainting and fainting types of reaction. In an analysis of 1050 cases (19), it was found that a majority, 53.3 per cent, of young men could be carried into an unconscious state in which the individual for 6 to 8 seconds sits glassy-eyed, deaf and irresponsive to signals and questions; while the remaining group, 46.7 per cent, were liable to faint. In the non-fainting type the systolic arterial blood pressure is

maintained at the normal or rises as anoxemia becomes extreme; diastolic pressure either maintains a level, rises a little, or falls gradually and moderately after the percentage of oxygen has been reduced to between 12 and 9 per cent. In the fainting type of reaction the systolic pressure may be maintained up to actual fainting or it may fall rapidly for several minutes before fainting occurs; and the diastolic pressure, after the oxygen has been reduced to the neighborhood of 15 to 12 per cent, undergoes a gradual fall which later suddenly changes to a very rapid fall that in 1 to 3 or 4 minutes eventuates in fainting. In a few cases a slowing of the heart rate is the first indication of oncoming syncope. This is soon followed by the fall in the arterial pressure. Ordinarily from 10 to 6 per cent of oxygen, comparable to an altitude of 20,000 to 30,000 feet, must be reached in order to produce the extreme vascular changes associated with fainting.

That in extreme anoxemia there is some degree of circulatory failure, especially in the fainting type of cases, is evident from our data on the diastolic arterial pressure, the venous pressure, the hand volume, and the blood flow in the capillaries and the hand. This is not due to a failure of the force of the heart but rather a failure of the venous return of the blood to the heart. Frequently in ordinary experiences a fall in diastolic pressure is associated with decreased peripheral resistance due to a dilatation of arterioles. However, in anoxemia it appears that the blood is to some extent diverted from the extremities to the trunk of the body and very likely from the periphery of the trunk to its interior. The evidence is clear that the hand volume decreases as anoxemia progresses. Apparently the tone of the peripheral vessels is not decreased as might be expected, but rather increased, with the result that the rate of flow of the blood through the capillaries is retarded. Associated with the peripheral vaso-constriction and the diverting of blood to the interior of the body is the fall in venous pressure. This seems to be due to a pooling of the blood in the splanchnic vessels because of a general relaxation of their arterioles. That splanchnic dilatation, with pooling, occurs seems evident from the fact that in large part abdominal pressure prevents the fall in venous pressure. From the observations on the diastolic arterial pressure, it appears that in many cases the relaxation of the splanchnic vessels occurs gradually; but that in fainting there may occur a sudden general relaxation of these vessels, which then causes fainting because of an inadequate venous return of blood to the heart.

That the above circulatory changes occur to some extent even in the non-fainting type of reaction is shown by the fact that in a large majority of all cases examined we have found some decrease in hand volume, a reduction in the rate of blood flow through the hand, a fall in venous pressure and a slight gradual fall in the diastolic arterial pressure. Because of a more adequate circulation the non-fainter can be given a degree

of anoxemia that renders inefficient the higher brain centers, while lower nerves centers are still able to maintain effective reflex muscular control. In the fainting type of reaction the centers of the medulla oblongata are profoundly affected before those of the fore-brain.

In the second type of circulatory reaction to anoxemia that we have studied, that in which a constant low level of oxygen is maintained, the interpretation of changes must also now be modified to conform to the new data. Lutz and Schneider (15), and Gregg, Lutz and Schneider (6) have shown the changes in the heart frequency and the arterial blood pressure to be as follows: The frequency of the heart beat increases as the available oxygen decreases, but the maximum rate usually does not occur simultaneously with arrival at the desired oxygen level. There is a lag of upwards to 5 or 10 minutes in the development of the maximum rate. After this the heart frequency may, after a period of maintained maximum, slowly retard, returning often nearly to the normal rate; or it may maintain the maximum rate throughout the hold; or it may slowly continue to increase throughout the entire period of the hold. The arterial blood pressure likewise changes so that during the first part of the hold the pulse pressure is increased. Later the pulse pressure tends to return to normal. The pulse pressure may be increased by a rise in the systolic pressure, but it is more frequently increased by a fall in the diastolic pressure. It is the return of these to normal that restores the pulse pressure to normal. In some individuals the diastolic pressure falls steadily and slowly throughout the entire period of the hold; and in some, after a period of slow fall, the decrease becomes rapid, so that the experiment is terminated because the subject faints.

In those cases in which the pulse rate and the arterial pressures return to or toward normal during the hold, some tendency to a circulatory failure is present at first, but is later overcome; and in those in which the pulse rate increases steadily and the diastolic pressure continues to fall throughout the hold period, the process of splanchnic pooling of the blood and the failure of the venous return steadily increase until the individual becomes ill and faint.

Since the evidence indicates that the output of the heart does not increase and that the venous return to the heart becomes subnormal during acute anoxemia, it becomes necessary to look upon the increase in the frequency of the heart beat and the diastolic arterial blood pressure fall as symptoms of distress rather than means of compensation to low oxygen.

SUMMARY

1. The circulatory effects of a gradually developed extreme anoxemia and of a constant moderate anoxemia are considered. Under the first condition there is an augmentation of the rate of the heart beat during

oxygen reduction. Acceleration is slight in the early stages, becomes more pronounced at 15 to 12 per cent oxygen, and may be profound as the limit of endurance is approached, which is usually between 7 and 6 per cent. Under the second condition, the frequency of the heart beat increases as the available oxygen decreases and continues to increase for upwards of 5 or 10 minutes after the oxygen level is reached. Afterwards, during the period of oxygen level, the heart rate, after a period of maintained maximum, may slowly retard; it may remain constant; or it may accelerate still more. Under favorable circumstances the rate retards.

2. The arterial blood pressure, under the first condition, may follow either of 2 courses. In one the systolic pressure is maintained or gradually rises, and the diastolic pressure is maintained or falls gradually and moderately as the anoxemia becomes extreme; while in the other course, the systolic and diastolic arterial pressures begin to fall rapidly sometime after the inhaled oxygen falls below 10 per cent. Under the second condition of anoxemia the arterial pressure changes, during the first part of the oxygen level period, increase the pulse pressure, either by a rise in the systolic or a moderate fall in the diastolic pressure. Later in a good type of reaction the pulse pressure returns to or toward normal, but in a poor type it may further increase by a steady and pronounced fall in the diastolic pressure.

3. The capillary blood pressure is not affected by anoxemia. The flow of blood through the capillaries, in some cases of extreme anoxemia, is gradually retarded; while the blood passes from a homogeneous to a granular appearance, and sometimes to a clumped condition of the red blood corpuscles.

4. There is a fall in the venous blood pressure which ordinarily begins when the oxygen approaches 16 per cent. The fall is slight at first and becomes more pronounced at between 12 and 10 per cent. The fall in venous pressure is, at least in part, due to a dilatation of the splanchnic blood vessels.

5. The hand volume usually decreases. The reduction often begins around 15 per cent of oxygen, but is most marked after 9 per cent is reached.

6. The blood flow through the hands, as determined by Stewart's hand calorimeter, was decreased in 76 per cent of all tests when the inhaled oxygen had been reduced to 13 per cent. At 7 per cent of oxygen the mean flow in a group of 29 rebreathing experiments retarded 32.8 per cent.

7. Attempts to determine the minute-volume output of the heart indicate that the output is not increased during anoxemia, and that it may sometimes be decreased. The stroke-volume of the heart is frequently decreased.

8. It is concluded that the circulatory changes in anoxemia do not serve as a means of compensating to a lack of oxygen, but that they may be interpreted as signals of distress.

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THE EFFECT OF EXERCISE ON THE DISTRIBUTION OF CORPUSCLES IN THE BLOOD STREAM[1]

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The effect of exercise on blood cell production and destruction has been studied experimentally from several points of view. Broun (1), working with dogs, found that the rate of red cell destruction increased after exercise if the animals had been inactive for some time, but that there was no marked change when the animals had been leading an active life. C. K. Drinker, K. R. Drinker and Kreutzmann (2) approached the problem from a different point of view. They varied the amount of blood flowing through the bone marrow capillaries of dogs, simulating, to some extent, the circulatory variations associated with exercise in other organs, as Krogh (3) showed to be the case with muscles. Both exercise (2, p. 263) and nerve section (2, p. 268) (dilatation of capillaries) as well as perfusion of the marrow itself at different rates and tensions failed to materially increase the nucleated red blood corpuscles in the peripheral circulation. Increases in the number of leucocytes in the peripheral circulation as a result of exercise have been described in both animals and man by numerous authors. Shultz (4), Burrows (5), Schneider and Havens (6), Hawk (7), Zunz and Shumberg (8) and Larrabee, Tileston and Emerson (9) report varying, but definite increases in the white cell count in the peripheral capillaries. An opportunity to study the effect of violent, prolonged exercise on the blood corpuscles of human beings was afforded by seventeen men who ran a Marathon race of 26¼ miles in between 2½ and 3 hours. The present paper deals with the results of the study of the blood in these men.

MATERIAL AND METHOD. The present study is limited to observations of the formed blood elements in supravitally stained blood films made from each of the runners directly before and very soon after the race. The blood was taken from the ear capillaries and films were made on glass

[1] This paper is no. 39 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed, in part, by a grant from the Proctor Fund of the Harvard Medical School, for the study of chronic diseases.

cover slips upon which a solution of brilliant cresyl blue had been dried. The preparations were then counterstained with Wright's stain, after the method of Cunningham (10). The differential counts of the white blood corpuscles were made from 200 cells in representative fields in the pre-race blood films and from 300 cells in the post-race preparations. Because it was not feasible to make blood volume determinations, the red and white cell counts per cubic millimeter were not made, as they give but little information by themselves under these conditions.

The study does not take into account the effects developing some time after the contest when compensation processes set in, the changes observed being those developing during the 2½ to 3 hours of constant exercise. Practically all, with the exception of the winner and two others, were almost completely exhausted by the race. In this respect, several who did not finish the race because of complete collapse, were comparable to the others who ran the full distance.

Observations. In the blood specimens taken before the race, considerable variation was noted in the number and different kinds of white cells. Table 1 shows the average differential counts before the race as well as the extremes. Estimating, from the pre-race blood smears, the approximate number of white blood cells and blood platelets, 29 per cent of the seventeen individuals started with a moderate increase in the number of leucocytes and 58 per cent showed a moderate increase, above normal, of their blood platelets, which, however, were of normal character (table 2). No gross abnormalities were encountered in the red blood corpuscles before the race, although 53 per cent of the athletes showed an increase of their young red blood cells (the granule red cells (11) and reticulated red cells) of from 1 to 6.4 per cent above an average normal of 3 per cent.

In the blood taken after the race, the most striking alterations in the cellular elements were in the polymorphonuclear neutrophile leucocytes and in the blood platelets. These elements showed both an absolute and a relative increase in every contestant. Larrabee, Tileston and Emerson (9), in studying the leucocyte numbers in marathon runners under somewhat similar condition, found 14,200 to 27,000 leucocytes per cubic millimeter. No figures have been found giving the platelet numbers. From the data in table 1 it is evident that the average increase of the polymorphonuclear neutrophile leucocytes after the race was 20 per cent greater than the number taken before the runners started. In one case after the race these cells reached as high as 94.7 per cent of the total leucocytes. In all of the cases, the polymorphonuclear neutrophile leucocytes appeared to be of the mature, adult type, with numerous clear-cut lobes in the nucleus. There was a marked relative decrease in the percentage of the other forms of white cells, with the exception of the transitional type of large mononuclears. The round nuclear type of large mononuclear

leucocytes, however, were greatly reduced in percentage numbers, as were the large lymphocytes. Since the total number of white cells per cubic millimeter of peripheral blood became increased, it is evident that the absolute numbers of the transitional type of large mononuclears increased while the round nuclear types of large mononuclears and large lymphocytes either remained the same in absolute number, or possibly diminished.

TABLE 1

Cellular composition of the blood of 17 marathon runners before and after the race

TYPE OF BLOOD CELL	BEFORE RACE		AFTER RACE	
	Extremes	Average	Extremes	Average
	per cent	*per cent*	*per cent*	*per cent*
Polymorph. neutrophiles	45.0–78.0	61.85	75.0–94.7	81.9
Polymorph. eosinophiles	0– 3.0	1.0	0– 0.3	0.035
Polymorph. basophiles	0– 2.0	0.5	0– 0.5	0.1
Large lymphocytes	2.0–14.0	7.9	0– 7.0	1.9
Small lymphocytes	8.0–28.5	16.3	1.3–15.4	4.89
Large mononuclears	.0–11.0	2.2	0– 2.7	0.56
"Transitional" mononuclears	3.0–23.0	9.7	2.0–16.7	6.6
Granule red cells	1.4– 6.6	3.0	1.8– 6.8	3.7
Reticulated red cells	0.4– 4.7	2.0	0.5– 4.0	1.4
Total young red cells	2.6– 9.4	5.0	2.5– 9.0	5.1
Megakaryocyte nuclei	0	0	0– 0.5	0.1

TABLE 2

Percentage numbers of individuals having equal or increased numbers of leucocytes and blood platelets before and after a marathon race

The increases in numbers of leucocytes and blood platelets were estimated from the blood films and were both relative and absolute.

	PERCENTAGE OF CONTESTANTS	
	Before race	After race
	per cent	*per cent*
Normal number of leucocytes	71	0
Increased number of leucocytes	29	100
Normal number of platelets	42	0
Increased number of platelets	58	100

The red blood corpuscles, in contrast to the white cells, did not show any definite changes in the percentage number of their different types. As no total counts were made in the present study, no definite statement can be given as to their absolute number per cubic millimeter. The blood films did not show any evidence of fragmentation of the red cells after the race, nor were there any microcytes. Table 1 shows that the average percentage

of young red blood cells before and after the race was practically the same. Only one nucleated red cell was encountered in all the blood films studied.

The increase in platelets was marked after the race, the number being estimated at about 3 to 5 times the number normally found in blood films. No exact quantitative data were recorded. Table 1 shows that 58 per cent of the contestants had an increased number of platelets before the race; all had an increased number after the race. One man showing an increased number before the race did not show a material change after the race. Many of the platelets were larger than normal, with more discrete granules in a clear, hyalin matrix. In three of the cases a large proportion of the platelets were of this type. In blood films made from five of the athletes after the race, a few structures resembling megakaryocyte nuclei were noted.

Blood was drawn from the contestant's arm veins before and after the race, for chemical study. The results are published by Levine, Gordon and Derick (12). At that time it was noted that the blood clotted much more quickly after the race than before. The time of clotting, however, was not studied quantitatively. It seems probable that there is a relationship between the increased platelet count of the blood and the increased speed of clotting.

Discussion. The study of the results of the blood examinations shows that after the violent exertion but few young cells were recognized. The change in the number of leucocytes, especially the polymorphonuclear neutrophiles, was apparently not due to a change in blood volume, as the other cells were not increased in corresponding ratio. The comparative absence of young forms among the white cells, and the lack of increase in young forms of red cells, suggests that the increased number of white cells in the peripheral circulation came, not from a fresh supply from the bone marrow, but from a redistribution of the circulating cells. This results in the appearance in the peripheral circulation of adult cells, which had been concentrated in the internal blood vessels, much as the "pseudo-crises" of normoblasts, which appeared in Drinker's (2) dog experiments after "stirring up" the circulation, after exercise, hemorrhage or infusion. Schneider and Havens (6) take the same view, holding that their observations of an increase in the number of red cells per cubic millimeter of blood from 3.2 to 22.0 per cent after exercise were explainable on the basis of the throwing into the circulation of large numbers of cells that were ordinarily side-tracked or inactive. Krogh's (3) direct observations show that stagnant cells may be present in even some of the more peripheral vessels. It is probable that such a process also accounts for the increase in platelets. However, the appearance of structures suggesting megakaryocytes, with the changes in the appearance of some of the platelets, makes it possible that, if any new structures left the bone marrow in appreciable quantities during the exercise, they were probably the platelets.

The increased number of young red cells before the race suggests that long training may raise the threshold of cell liberation from the bone marrow—a process which may constitute a part of the difference between a trained and untrained athlete. However, the tension and excitement preceding the contest may have been factors. The lack of further increase in young red cells parallels the experimental observations of Drinker et al. (2, p. 261) who found that exercise, coupled with marrow hyperplasia, did not cause an increase (in the experimental animals) of normoblasts. The absence of microcytes, poikilocytes or other forms of red cells associated with blood destruction was a striking and unexpected feature, although in accord with Broun's (1) observations on dogs which had led an active life.

The data of before and after the race were studied to see if there was any correlation between the order in which the contestants finished and the blood findings. No parallelism was discoverable, nor were other factors, such as age, general physique or nationality determining criteria.

The study, as a whole, confirms the experimental work of C. K. Drinker, K. R. Drinker, and Kreutzmann as well as the observations by other authors (4), (5), (6), (7), (8), (9), and strengthens the view that the difference in cellular composition of the blood after a period of exercise is the result of the "stirring up" into the general circulation of blood corpuscles temporarily "stored" in less active areas.[2]

SUMMARY

1. All of 17 individuals running a marathon race of 26¼ miles in 2½ to 3 hours, showed a relative and absolute increase in the number of their polymorphonuclear neutrophile leucocytes and blood platelets in the peripheral circulation.

2. The character of the red cells and the relative percentage of their immature forms remained practically unchanged in the peripheral circulation during the exercise.

3. There was no definite evidence of newly formed or immature cellular elements (with the possible exception of the blood platelets) being added to the circulation during the period of exercise.

4. The change in the number of white cells and blood platelets is probably the result of a more thorough mixing of the blood of the internal and peripheral blood vessels, with a redistribution of its elements.

5. An increased number of young red cells and, in some cases, blood platelets and white cells in the blood of the athletes was observed before the race. This suggests a possible relationship between the previous period of training and the slightly lowered threshold of blood cell delivery.

[2] The assistance of Drs. Benjamin Brock and Raymond Reitzel in taking the blood films is acknowledged with appreciation.

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THE EFFECT OF RESPIRATION ON THE VENOUS PULSE AS STUDIED BY THE ELECTROPOLYGRAPH

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The present investigation was undertaken to study, by means of the writer's electropolygraph (1), the influence of the respiratory movements on the venous pulse. The principle of the electropolygraph may be briefly outlined.

An open receiving cup is applied over the vein and the pressure changes in the latter are transmitted by a rubber tube to a tambour, the movements of the membrane of which cause oscillations of the diaphragm of a microphone. These oscillations induce changes in resistance of the carbon granules in the microphone which effect corresponding variations in the magnetic field of a solenoid. These variations cause corresponding movements of an armature, with pointer attached, which swings inside the solenoid. The excursions of the pointer are recorded on a smoked surface and afford an accurate record of the venous pulse.

In recording the arterial pulse the button of the microphone may be applied directly to the artery or the open receiving cup may be replaced by a glycerine tambour which is properly adjusted over the artery. Apart from this difference the method of recording the arterial does not differ from that used in the case of the venous pulse.

Physical examination of the thorax on each of the subjects revealed no abnormality.

Usually at the beginning of expiration there is a sudden rise in the venous tracing. This rise is maintained during approximately the first third of expiration and is followed by a gradual fall which is continued through the remaining part of expiration. At the beginning of inspiration there is a sudden fall in the venous tracing followed by a more gradual fall which lasts throughout inspiration. This is followed by the rise referred to above.

It is probable that the effects mentioned above can be explained by changes which occur in the thorax during respiration. The above rise in the venous tracing is typically shown in figure 2. In figure 1 the rise took place just before the end of inspiration. When the capacity of the

chest is increased in inspiration the intra-thoracic pressure is decreased from approximately −5 to −7 mm. of mercury to −30 mm.; that is to say, from 5 to 7 to 30 mm. less than the atmospheric pressure (760 mm. of mercury). The pressure outside the heart and large thoracic vessels is correspondingly diminished during inspiration, and produces its main effect (distention) upon the right auricle and vena cava. This increase in the rate of fall of pressure between the intra- and extra-thoracic great veins results in a proportionally more rapid flow of blood into the thorax (2). This increased flow causes a partial collapse of the thin-walled internal jugular vein over which the receiving cup is held. This collapse is evidenced by a fall in the venous tracing at beginning of inspiration. The extent of dilatation of the right auricle and superior vena cava being limited, there will be a time somewhere in the latter part of inspiration or beginning of expiration at which they have expanded to their full extent. The amount of blood drawn into the superior vena cava and right auricle on inspiration will be in proportion to the dilatation, and when dilatation ceases blood will continue to flow for a moment until any available space is used up. Therefore when the limit of expansion is reached the auricle and veins will not only be rigid from distention but also well filled with blood. If, now, the auricle contracts its pressure changes will be more forcibly transmitted to the internal jugular vein, there being little absorption of the impact by the rigid superior vena cava. The internal jugular vein being flaccid and also near the heart will expand considerably with the impact, resulting in an increase in the height of the venous tracing. (See fig. 1, *a'*.)

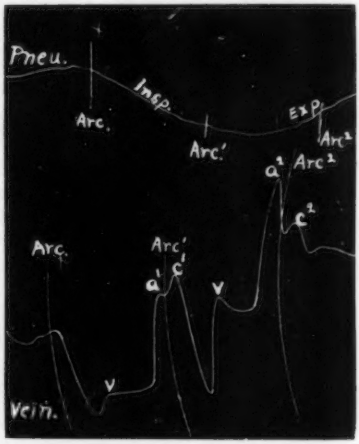

Fig. 1. Pulse records of the internal jugular vein obtained with the electropolygraph and simultaneous pneumatogram. *Arc*, *Arc*¹ and *Arc*,² corresponding arcs; *A*, A wave; *C*, C wave of MacKenzie; *Exp.*, expiration; *Insp.*, inspiration; *pneu.*, pneumatogram; *V*, V wave; *X*, depression X.

The time at which the right auricle and superior vena cava reach their limit of expansion and become completely filled is dependent upon the depth, the length of the respiratory act, and the rapidity with which the blood is passed on into the right ventricle.

As stated above, this limit of expansion and filling of the right auricle and superior vena cava may not take place before the beginning of ex-

piration. When this is the case the walls of the already full and distended right auricle and intra-thoracic veins will have extra support given them by the increased intra-thoracic pressure due to expiration. The above condition of the superior vena cava and right innominate veins makes them an efficient manometer whereby the auricular pressure changes are very effectually transmitted to the internal jugular vein.

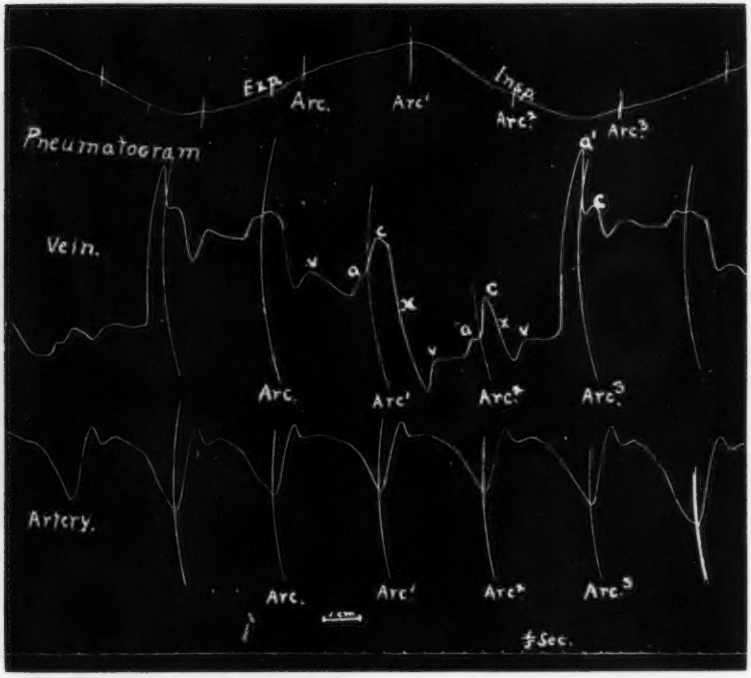

Fig. 2. Pulse records of the internal jugular vein and carotid artery obtained by means of the electropolygraph together with simultaneous pneumatogram. *Arc*, *Arc*^{1}, *Arc*,^{2} *Arc*,^{3} corresponding arcs; *A*, A wave; *C*, C wave of MacKenzie; *Exp.*, expiration; *Insp.*, inspiration; *P*, P wave of carotid pulse; *V*, V wave of jugular pulse; *X*, depression X of jugular pulse. Time in $\frac{1}{5}$ second.

The result is a very sudden rise in the venous tracing as is evidenced in figure 2.

As pointed out above, there are two factors which modify the venous pulse during inspiration, namely, *1*, pressure, and *2*, increased blood volume. During expiration the thoracic volume is decreasing, therefore the blood flow from the peripheral veins, which is one of the main fac-

tors increasing the blood volume in the auricle, superior vena cava and internal jugular veins, is decreased (2).

During the first two heart cycles occurring in expiration the extra blood drawn in during the previous inspiration serves to keep the blood volume in the right auricle and veins high. When, however, the extra blood is passed on into the right ventricle, which requires about two heart cycles, the auricle and veins are left with very little blood in them. This decreased blood volume is continued through the remaining part of expiration to the beginning of inspiration. The maintenance of distention of the internal jugular veins during the first part of expiration and the drop following are evidenced in the venous tracing, figure 2. It is further observed that the drop is continued into the following inspiration by the suction on the internal jugular vein due to increase in volume of the thorax.

It is evident that the above variations in the general forms of the venous tracing are brought about by changes in the individual waves. It will, therefore, be necessary to consider each wave separately.

The "A" wave being due to contraction of the auricle is very prominent whenever the auricular pressure changes are being well conducted to the internal jugular vein. As stated above, these conditions occur when the auricle and superior vena cava are well filled with blood and also when the walls of the veins are rigid from distention or by external support due to increased intra-thoracic pressure. This is well brought out in *A 1*, figure 1, and *A 1*, figure 2. In the former the rigidity of the venous wall is due to distention, and in the latter there is the added support of increased intra-thoracic pressure of expiration, an adequate supply of blood being present in both cases.

The "V" wave is increased in height by the same factors that increase the "A" wave. The rise "V" is due to the damming back of blood in the auricle, superior vena cava, innominate and internal jugular veins during systole of the ventricle. This results in distention of the internal jugular vein. Distention of the intra-thoracic veins is prevented by the increased intra-thoracic pressure caused by expiration. Hence, the less the distention of the intrathoracic veins the greater will be the distention of the internal jugular vein. Therefore at the beginning of expiration when the intra-thoracic pressure is rising and the veins are already well filled from the previous inspiratory act there will be a greater distention of the internal jugular veins before the A-V valves open and allow the blood to flow into the ventricle. This is well shown in figure 3.

The "C" wave is only slightly affected by respiration and therefore is comparatively higher than the other waves when the pressure in the thoracic cavity is low. In figures 1 and 2 it will be seen that where the venous tracing is low the "C" wave is not reduced in proportion to the other waves.

Piersol (3) observed the internal jugular vein in man during inspiration and expiration and to quote his own words "After the carotid sheath has been opened the vein will vary in appearance from a distended thin-walled tube perhaps half an inch in diameter, (expiration) to a flaccid ribbon-like structure with the walls apparently in contact (inspiration)." In the region of the inferior jugular bulb, over which area the receiving cup is placed, the carotid artery and internal jugular vein are not in close proximity (4). In view of these observations it is reasonable to conclude that a considerable portion of the impact from the carotid artery is transmitted to the receiving cup directly through the overlying sterno-cleido-mastoid muscle and not wholly by way of the internal jugular vein. Granting the above to be true, one can readily understand how collapse of the vein would allow the overlying muscle to come in closer contact with the carotid artery and thus produce a comparatively high "C" wave in the venous tracing. With the overlying muscle raised away from the carotid artery at varying distances by the vein it will be necessary for the artery to expand to varying degrees before it will be able to exert any force on the overlying muscle and thus produce a wave in the venous pulse. As the rise of the "P" wave of the carotid tracing extends over a period of $\frac{1}{6}$ second the beginning of the "C" wave of the venous pulse may be either synchronous with the beginning of the "P" wave or succeed it by as much as nearly $\frac{1}{6}$ second.

From figure 1 it is evident that the "C" wave following the high "A" wave is delayed. This "C" wave occurs at a time when the sterno-cleido-mastoid muscle is pushed farthest away from the carotid artery by the great distention of the internal jugular vein. On the other hand, the "C" wave following the low "A" wave is synchronous with the beginning of the "P" wave of the carotid pulse. This wave occurs at a time when the vein is lax and hence the sterno-cleido-mastoid muscle is in closer proximity to the carotid artery.

Experimental proof that varying the distance between overlying structures and a pulsating elastic tube alters the time relations of a tracing of the pulsations was furnished by the following experiment. An elastic tube into which fluid is forced by a sudden stroke of a pump was secured by two clamps. A cork was attached to each of the two tambours, which were placed side by side, the corks resting with equal pressure on the rubber tube. Each tambour was connected by rubber tubing to another tambour supplied with lever and writing point. Tracings were taken on a moving drum. When both corks rested with equal pressure upon the pulsating rubber tube the rises of the levers were found to be synchronous, but when one cork was placed 0.5 mm. from the tube there was a very appreciable delay in the rise of the corresponding lever. Tracings made in the above way are shown in figure 4.

The temporal relations of the "C" wave are also changed to a certain degree by varying the amount of pressure and method of application of the receiving cup. Greater pressure or placing the receiver more directly over the artery increases the corresponding height of the "C" wave.

It is evident that the greatest depression, *X*, occurs at the beginning of inspiration (fig. 2). MacKenzie (5) states that the fall *X* is due to three factors: 1, the relaxation of the auricle after its systole, 2, the dragging down of the auriculo-ventricular septum by the ventricular

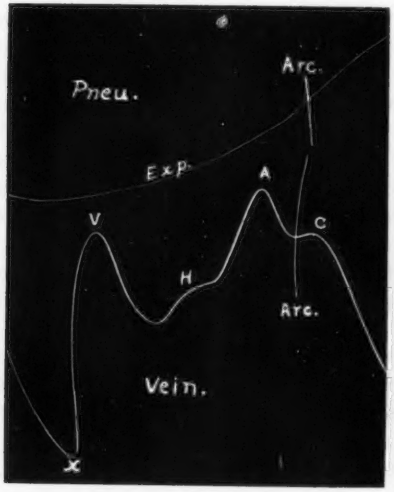

Fig. 3

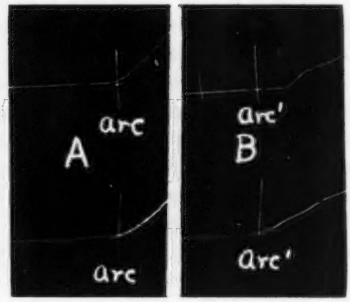

Fig. 4

Fig. 3. Pulse record of internal jugular vein obtained with the electropolygraph and simultaneous pneumatogram; *Arc*, corresponding arcs; *A*, A wave; *C*, C wave of MacKenzie; *Exp.*, expiration; *H*, H wave of Hirschfelder; *Pneu.*, pneumatogram; *V*, V wave; *X*, X depression.

Fig. 4. Tracings obtained by varying the distance of a cork attached to a tambour from a pulsating elastic tube; *A*, tracing made with both tambours resting with equal pressures on the tube, *B*, tracing made with tambour connected to top pen raised .5 mm. above the elastic tube; *Arc*, *Arc*^{1}, corresponding arcs.

muscle, enlarging the auricular cavity; 3, the diminished intra-thoracic pressure in consequence of the expulsion from the chest of the contents of the left ventricle. The present work shows that at the beginning of inspiration the depression *X* is markedly increased. The intra-thoracic pressure at this phase of the respiratory cycle is diminished and the intra-thoracic veins and auricles would accordingly tend to dilate. It therefore seems plausible that the increased depression *X* at this phase of the respiratory cycle can be accounted for by the addition of this factor to

those enumerated by MacKenzie. This accentuated drop is not so noticeable in the following cardiac cycle because by this time the venous tracing had already reached approximately its lowest level.

The "H" wave is most marked at the beginning of expiration. It will be noted in figure 3 that the "H" wave of Hirschfelder or "B" wave of Gibson is present. The "H" wave is not a prominent feature in the venous tracings of the subjects studied, but when it did occur it was always at the beginning of expiration. The presence of the "H" wave at this particular phase of respiration is no doubt due to the fact that at this time the vena cava and internal jugular veins are well filled from the previous inspiratory act, also their walls are well supported by the increased intra-thoracic pressure at this period. It is therefore evident that the conditions for the propagation of small waves from the auricle to the internal jugular vein are ideal. Another factor which was found to be essential for the production of the "H" wave was a rapidly moving drum thus giving ample space in the tracing to bring out the wave. This may be responsible for the statement that the "H" wave is more often found in slow-beating hearts.

In recording venous tracings during active respiratory movements the possibility that the changes in the tracing are due to movements of the sterno-cleido-mastoid muscle directly under the receiver must be considered. This possibility was excluded by making tracings from the areas surrounding the inferior jugular bulb. In the tracings obtained from the above areas it was found that if the receiver was held stationary and the clavicle fixed there were no movements of the recording pen.

As stated above the "C" wave is only slightly affected by respiration. This wave is due to pressure changes outside the thorax (5). It is therefore further proof that the changes described are of intra-thoracic origin.

SUMMARY

1. Respiration causes a rise and a fall in the venous tracing; the rise commences in the latter half of inspiration or the beginning of expiration and is continued over about the first two heart cycles during expiration; a fall followed the elevation and is continued to the next rise.

2. The height of the "A" and the "V" waves is increased when respiration aids transmission of auricular pressure changes to the internal jugular vein.

3. The height of the "C" wave is little affected by respiration, but its temporal relations are changed.

4. The "H" wave is more prominent and often only present at the beginning of expiration.

I wish to express my thanks to Dr. F. R. Miller and Dr. N. B. Laughton for their kind criticism and suggestions during this work.

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VACCINE FEVER IN RABBITS RENDERED POIKILOTHERMOUS BY CERVICAL CORD TRANSECTION

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Balcar, Sansum and Woodyatt (1) injected intravenously into dogs concentrated solutions of glucose and observed an elevation of the animal's rectal temperature. The temperatures observed were quite high and were frequently associated with definite chilling. After a review of the literature on sugar and salt fevers, the authors concluded that probably all the facts of these fevers could better be explained on a basis of tissue dehydration than on the basis of other existing theories. A physicochemical theory of fever then was suggested which attributed the rise in temperature to an absence of "free water" in quantities adequate for heat dispersal. The height of the temperature, other things being equal, would give an index of the state of tissue hydration. This "free" water might actually be removed from the body in the form of urine, as in the experiments cited, or on the other hand it might enter into combination with the colloids and thus be rendered unavailable for heat dispersal. While such a conception of fever does not necessarily eliminate a heat-regulating center with all of its nervous connections from an important place in the febrogenic mechanism, yet it finds the individual cells, whose known range of water traffic is large, a more plausible site for the febrile reaction. These investigators therefore submitted dogs rendered poikilothermous by cervical cord transection to glucose injections and found that these animals also reacted with a fever, when the water loss was extreme. So, if this theory, with its implication that the site of the febrile reaction is peripheral, is to be extended to infectious fevers, it must be shown that animals robbed of their heat-regulating mechanism can still show infectious fever.

Freund and Grafe (2) state that in animals rendered completely poikilothermous such agents as trypanosomes and swine pest bacilli increase neither the metabolism nor the body temperature, although they cause an early death in the inoculated animal. Smith (3) found that anaphylactic temperatures did not result after section of the cord in either the dorsal or upper lumbar region. Cervical cord animals were not used.

It is evident that none of Smith's animals were poikilothermous in the sense defined by Freund and Strassman (4) and Freund and Grafe (2), and so the experiments are not pertinent to the question. Judah Jona (5) injected into dethalamate rabbits a suspension of B. coli dried in sodium sulphate solution (pyrogen). Since the injections were made shortly after the decerebration, the author himself states that his animals were more or less in a state of shock and that consequently his results were probably not so valuable for this reason. Citron and Leschke (6) have apparently produced very satisfactory physiological preparations of dethalamate animals which survived a number of days under artificial feeding. These animals were infected with trypanosomes, streptococci and staphylococci, and died without developing a fever. More recently Schmitt and Sansum (7) have reported vaccine fever in dogs with cervical cords transected. The present work is on obvious development of the fever problem as outlined in the earlier communication (1) from this laboratory. The importance of the subject justifies us in this study, although one of the joint authors of the earlier communications has already published independent data on this subject.

EXPERIMENTAL METHODS. *Test animal.* Rabbits were chosen as test animals. They were rendered poikilothermous by cervical cord section. The reasons for the choice of this animal and the method followed in the section have been stated elsewhere (8). These animals return to a good nutritional condition in three to four days following the cord section and they will live easily ten to fourteen days. If before the operation they are not allowed to mingle with the stock rabbits and acquire respiratory infections, they can be kept alive almost indefinitely. It must be emphasized that these animals were good physiological preparations, that they had recovered from the shock of an extraordinarily severe operation, and that they were in a satisfactory nutritional state.

Maintenance of body temperature. The body temperature was maintained by wrapping the animal in covers (cotton blankets) and at times applying external heat in the form of an electric light. The head was so adjusted that room air could be easily breathed, and thus eliminate any question of a rise in temperature being due to an increased atmospheric humidity. This method of maintaining the body temperature offers some advantages over a thermostat arrangement used by other investigators. If the heat production within the animal is increased we have an arrangement of poor conductors that tends to resist its dispersal and to favor an establishment of a proportionately higher temperature. If one considers the thermostat arrangement such as Freund and Grafe (3) and others have used, the relations are different. Here the thermostat was ventilated and the container for the rabbit was immersed in a water jacket maintained at a fixed temperature with slight variations.

The ventilating air would then act as a breeze, which would favor heat dispersal. With the elevation of the animal's temperature there would be established both a temperature and moisture gradient. Even if there were no elevation of the animal's temperature there would still be a significant moisture gradient unless the ventilating air had a comparatively high humidity. The water jacket to the animal cage would not furnish a constant source of heat. As heat is added to it from the inside (rabbit) the thermo-regulator would cut down the supply of heat furnished from the outside heating units. The rabbit's heat would then be used to keep the water bath at a constant temperature. Such an apparatus would be so successful in dissipating heat, that it is doubtful if an increased heat production in the rabbit would express itself in a temperature rise.

Proof of poikilothermous state. This was conducted simply by changing the surroundings of the animal. A rabbit because of the small body mass reacts rather readily, so that temperature changes manifest themselves at relatively short intervals.

Vaccines. The Park Davis preparation of typhoid-paratyphoid vaccine (preparation "Bio 441") was used. The quantity of fluid injected did not in any case exceed 2 to 2.5 cc. The injections were made intravenously and intramuscularly or subcutaneously.

EXPERIMENTAL RESULTS. *Proof of the poikilothermous state.* It is of course of prime importance to show that the methods employed gave poikilothermous animals during the periods in which the experiments were conducted. For this reason table 1 has been compiled. It shows that in animals with cervical cord transactions a poikilothermous state existed from the 1st to the 11th day. At no time was an attempt made to determine the lowest range of temperature that could be secured and still have the animal recover satisfactorily. It was feared that such procedures would facilitate the development of pneumonia. However, the protocols have been examined for the temperature ranges which occurred during these studies. The low temperatures of 31.9°C. in one case, and 33.3°C. in three cases were observed. The other animals showed ranges from 34° to 36°C. upward. The highest recorded temperature was 43°C. In two animals a temperature of 42.2°C. was present. In the remainder, the external heat and covers were so adjusted as to keep the range more nearly that found normally in rabbits.

Effect of food. Freund and Strassman (4) have reported that animals with dorsal cords transected usually, though not always, reacted to feeding with a temperature rise. The animals with cervical cord transections, however, routinely reacted with an increase of 1.5°C. in 5 to 6 hours after feeding. In table 2 it is seen that febrile reactions occurred as early as the second hour. This early reaction might be explained by

TABLE 1

Proof of poikilothermous state

ANIMAL	DAYS POST-OPERATIVE	SITE OF TRANSECTION	VARIATIONS IN ANIMAL'S TEMPERATURE		DURATION OF EXPERIMENT	EXPERIMENTAL CHANGES
			Temperature	Change		
					hours	
III	2	D I	39.1–37.6	1.5	3.0	External heat removed. Covers maintained in place
III	2	D I	38.9–38.0	0.9	2.0	External heat applied. Covers maintained the same
IV	1	D I C VII	40.0–36.1	3.9	3.0	External heat removed. Covers maintained
IV	2	D I C VII	39.4–38.8	0.6	2.5	External heat maintained. Covers removed
VI	9	C VII	40.0–38.6	1.4	2.5	Animal moved out of sun into cooler place
VI	9	C VII	38.6–37.1	1.5	1.0	Fan turned directly on animal
VI	11	C VII	38.3–31.9	6.4	30.0	Covered in cool room for 30 hrs. Fed, no external heat. Temperature at end of this time
VII	5	C VII	41.6–38.1	3.5	3.0	External heat and covers removed, placed in breeze
IX	9	C VII	38.2–36.4	1.8	3.5	Covers maintained, placed in window
X	8	C VII C VI	38.0–36.6	1.4	3.0	Covers maintained, placed in open window
XI	7	C VII C VI	37.2–34.4	2.8	3.5	Covers maintained, placed in open window
XII		C VII	39.0–33.3	5.7		No specific experiment. Temperature ranges under laboratory conditions
XIII	2	C VII C VI	40.6–39.4	1.2	1.5	Fan turned on without change of external temperature
XIV		C VII	39.4–36.4	3.0		No specific experiment; temperature ranges under laboratory conditions
XVII		C VI	39.7–37.2	2.5		No specific experiment; temperature ranges under laboratory conditions
XX	4	*	39.9–38.3	1.6	3.0	External heat removed. Coverings maintained
XXI	5	C VII	38.9–36.1	2.8	1.0	Fan applied. Covers removed
XXI	6	C VII	39.6–34.4	5.2	2.0	Put in ice box protected with a blanket

* Animal discarded by mistake before autopsy was done.

It was difficult to estimate exactly the environmental temperature especially where external heat was applied, so no exact temperature ranges are presented.

a retention of heat produced in mastication and other motor phenomena of digestion. The rises which are recorded at the end of 7.5 to 12 hours are more pronounced and probably represent the full fuel effect of the food ingested.

Vaccine fever. The intravenous method of injection was used on four animals with negative results. Shortly after the injections they showed a drop in temperature, developed a diarrhea, manifested symptoms of profound prostration, and died at varying intervals depending on the dosage of vaccine. The inability of these animals to resist the intravenous injections of vaccine was in sharp contrast to that of the normal animals. Even small doses given by this route were extremely toxic. Figures 1 and 2 show the temperature reactions in the cases of rabbits III and VII.

TABLE 2

Effect of food on rectal temperature of poikilothermous rabbits

ANIMAL	SITE OF TRANSECTION	TEMPERATURE			DURATION OF EXPERIMENT IN HOURS	DAYS POST-OPERATIVE
		Before feeding	After feeding	Increase		
III	D I	38.3	39.8	1.5	8.0	1
VI	C VII	33.8	36.7	2.9	2.5	12
VI	C VII	37.9	40.0	2.1	7.5	13
VII	C VII	38.1	39.8	1.7	7.5	6
IX	C VII	36.7	30.0	3.3	8.0	2
IX	C VII	37.2	38.4	1.2	2.0	4
X	C VII C VI	38.3	39.1	0.8	2.0	3
XI	C VII C VI	38.3	39.3	1.0	3.5	4
XXI	C VII	36.1	39.7	3.6	12.0	5

External environment remained the same throughout the experiment.

Attention is called to the poikilothermous state of rabbit III as manifested in the fall from 39.1° to 38°C. on the withdrawal of heat, and the rise resulting from the return of the same external heat. In this case the level was brought back to 38.9°C. because this was more nearly the rabbit's normal temperature, and it was expected, therefore, that this would be the most favorable level for the development of fever.

Because of the toxicity of the intravenous injections, the intramuscular and the subcutaneous routes were tried. The positive results are summarized in table 3. Figures 3, 4, 5 and 6 show the character of the response. Similar curves were obtained in four other experiments. These have been omitted to avoid repetition of data. In brief the characteristics of the febrile response may be summarized:

a. The reaction can be stated from a wide range of levels (39.3° in case of rabbit X and 36.1° in rabbit XII).

b. The temperature increase varied between 1.2° and 2.9°.

c. The onset of a definite rise began as a rule in 1.5 to 2 hours.

d. The high constant temperature was reached in from 3 to 7 hours (average of 5.6 hours), and it persisted over 12 hours in seven experiments. The eighth experiment was terminated short of this time.

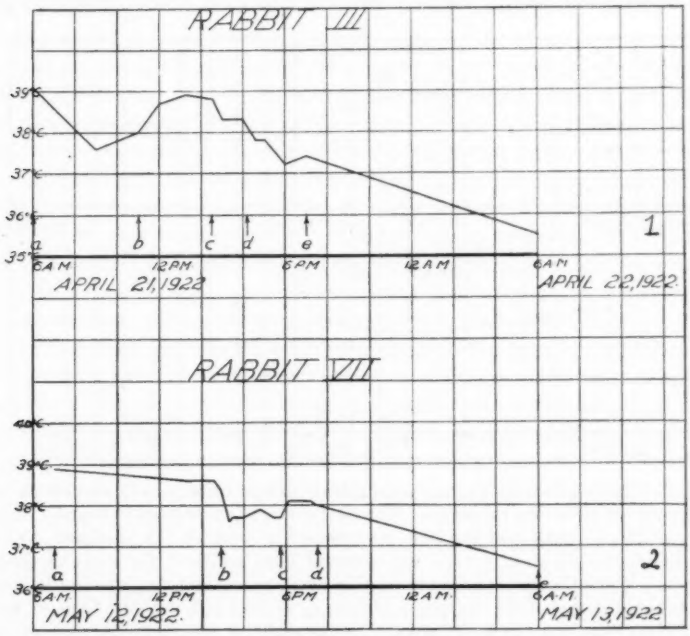

Fig. 1. Showing the effect of repeated intravenous injections of vaccine on rectal temperature of rabbit III. The temperature is expressed in degrees (Centigrade) in the ordinates and the time in hours in the abscissae as indicated below the line. *a*, Lights turned off but covering maintained; *b*, lights turned on again; *c*, 735 millions bacilli injected intravenously; *d*, 2500 millions bacilli injected intravenously; *e*, additional covers and external heat applied.

Fig. 2. Showing the effect of repeated intravenous injections of vaccine on rectal temperature of rabbit VII. The temperature is expressed in degrees (Centigrade) in the ordinates and the time in hours in the abscissae as indicated below the line. *a*, lights and covers adjusted for the experiment; *b*, 833 millions bacilli injected intravenously; *c*, 1,250 millions bacilli injected intravenously; *d*, covers left same for the night; *e*, experiment terminated.

e. The animals on the day following the experiment usually reacted to food with a fever, which was not so pronounced.

f. Forty-eight hours after the experiment the temperature levels had again returned to the fasting state.

Negative results. The question arises whether any negative experiments were obtained after the intramuscular injection of the vaccine. In four of the animals several days after the first injection, the experiments were repeated with negative results. The rabbits were emaciated, had eaten poorly after the first experiment, and were now no longer in a good nutritional state. Their condition might be characterized as pre-

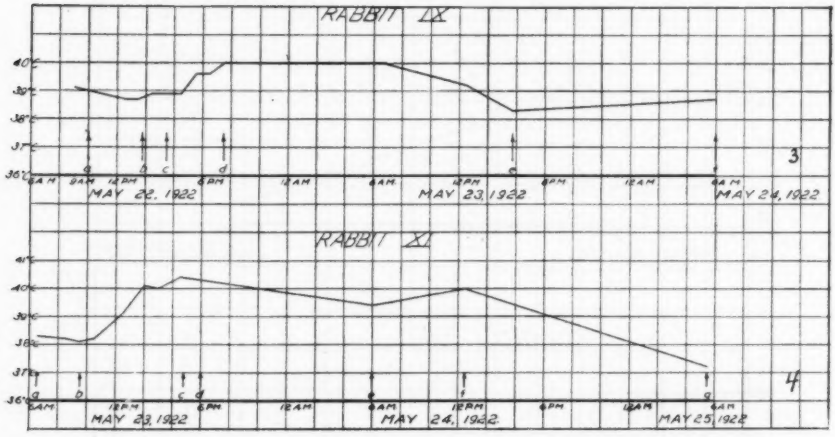

Fig. 3. Showing the effect of repeated intramuscular injections of vaccine and the administration of food to rabbit IX. The temperature is expressed in degrees (Centigrade) in the ordinates and the time is expressed in hours in the abscissae, as indicated below the line. *a*, covers and external heat adjusted for the experiment; *b*, injected 1000 millions typhoid-paratyphoid intramuscularly; *c*, injected 1000 millions typhoid-paratyphoid intramuscularly; *d*, animal left without food or water and without changing the surroundings for the night; *e*, animal fed during the next two hours; *f*, temperature height some 12 hours after feeding.

Fig. 4. Showing the effect of a single intramuscular injection of vaccine and the administration of small amounts of food in the case of rabbit XI. The temperature is expressed in degrees (Centigrade) in the ordinates and the time is expressed in hours in the abscissae, as indicated below the line. *a* covers and the external heat adjusted for the experiment; *b*, injected intramuscularly 2000 millions typhoid-paratyphoid; *c*, gave 30 cc. water with medicine dropper; *d*, animal left without food or water and without changing the surroundings for the night; *e*, animal ate poorly so that the quantity of food taken was small; *f*, this represents the high point of this amount of food stimulation; *g*, experiment terminated.

moribund. None of them survived the second injection longer than 16 hours. These results only emphasize the value of the limitations that were set upon the test animal at the outset. The test animal must be a good physiological poikilothermous preparation, and not one suffering from shock, or one poisoned with ether, or one whose storehouses of fuel have been depleted to the moribund level.

DISCUSSION. A careful search of the literature has been made for studies on vaccine fever in poikilothermous animals. Schmitt and Sansum (7), continuing work begun in this laboratory, have reported such investigations. They report fever in dogs shortly after a cervical cord transection with repeated intravenous injections of typhoid-paratyphoid vaccine.

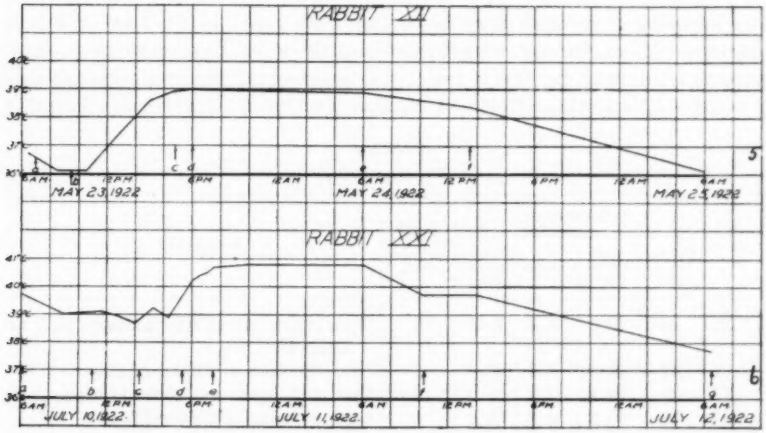

Fig. 5. Showing the effect of a single intramuscular injection of vaccine and the administration of a small amount of food in case of rabbit XII. The temperature is expressed in degrees (Centigrade) in the ordinates and the time is expressed in hours in the abscissae, as indicated below the line. *a*, covers and external heat adjusted for the experiment; *b*, injected 2000 millions typhoid-paratyphoid intramuscularly; *c*, gave 30 cc. of water with a medicine dropper; *d*, animal left without food and water, and without changing the surroundings for the night; *e*, animal ate poorly so that the quantity of food taken was small; *f*, covers adjusted for the day and remained unchanged through the night.

Fig. 6. Showing the effects of repeated intramuscular injections of vaccine and the adminsitration of food in the case of rabbit XXI. The temperature is expressed in degree (Centigrade) in the ordinates and the time is expressed in hours in the abscissae as indicated below the line. *a*, external covers and heat adjusted for the experiment; *b*, administered 30 cc. water with medicine dropper; *c*, injected 2500 millions typhoid-paratyphoid intramuscularly; *d*, injected 1000 millions typhoid-paratyphoid intramuscularly; *e*, animal left without changing the experimental conditions for the night; *f*, animal ate greedily.

The failure of Freund and Grafe (2) and of Citron and Leschke (6) to elicit fever by infecting poikilothermous animals with bacteria and trypanosomes does not negate results obtained by vaccine injections. Several explanations might be offered for their negative results. In the first place attention has already been called to their thermostat arrangement which would certainly favor heat dissipation. In the second place

their animals may have been depleted of their glycogen stores and thus may not have had adequate fuel readily available. No mention is made in their technique that the importance of this factor has been considered. It is a definitely appreciated fact among clinicians that a fall in temperature occurring in the course of such a wasting disease as tuberculosis may be considered not as a sign of improvement but as one of terminal exhaustion. In the third place the doses of toxic material developed by the infecting organisms may have been too great to produce a febrile reaction. Thus in our experiments intravenous injections of vaccine were not followed by fever, but by a lowering of the temperature and a rapid exitus of the rabbit. A clinical analogy for this also is found in

TABLE 3

Vaccine fever

ANIMAL	TEMPERATURE				DAYS POST-OPERATIVE	REMARKS
	Before injection	After injection*	Change	Next morning		
IX	38.7	40.0	1.3	40.0	6	Two injections of 1000 millions at 2 hr. intervals
X	39.3	40.6	1.3	40.4	5	One injection of 1000 millions
XI	38.1	40.4	2.3	39.4	5	One injection of 2000 millions
XII	36.1	39.0	2.9	38.9	4	One injection of 2000 millions
XVII	38.6	39.8	1.2	†	4	One injection of 2500 millions
XX	37.2	39.7	2.5	39.7	4	Two injections of 2500 and 1000 millions at 3 hr. intervals
XXI	38.7	40.8	2.1	40.8	3	Two injections of 2500 and 1000 millions at 3 hr. intervals
XXI	38.4	40.4	2.0	40.0	7	Two injections of 2500 millions at 3 hr. intervals

*Represents highest level attained.
†No record.

the very severe streptococcic infections. Such an infection in "the young" may run its course with a very slight febrile response and in "the aged" such cases are regularly found without fever. The clinician has come to look upon this afebrile state as simply evidence of the virulence of the infecting agent. For these reasons one should not consider that the results of these authors establish the conclusion that poikilothermous animals, along with their loss of ability to regulate bodily temperature to the environment, have also lost the power of febrile response to bacterial intoxication.

Kennaway and Pembrey (9) concluded from their studies on mice and rabbits with their cords sectioned in the dorsal region that the animals behaved as essentially normal animals in that portion of the body above

the section, and as poikilothermous animals in that portion below the section, that the effect on the respiratory exchange, the respiratory quotient and the temperature was a resultant of the metabolic activities of the two portions of the body. On such a basis one might say that the febrile reaction, in rabbits with cervical cord transection, is due to the response of this normal anterior portion of the animal. If such a view is adopted, we then ask ourselves what is comprised in the anterior normal portion of the rabbit, which is innervated by the nerves originating above the 6th cervical segment? This normal anterior non-poikilothermous region will be found to comprise the head and neck. The posterior poikilothermous region will comprise the body, the extremities and the ears of the animal. The former portion has therefore a very small mathematical value as compared with the latter. Certainly any febrile response arising in the normal portion, which contains relatively little of the animal's musculature, would be insignificant. It is clear therefore that this demonstration of a vaccine temperature in rabbits with cervical cords transected, does not conflict with any data published up to the present time. It is further evident that while the removal of a heat regulating center may modify the character of the febrile response and may make it necessary to adopt certain precautions in eliciting fever, this removal does not abolish absolutely the response. Whether the ultimate seat of the febrile response is to be found in less highly integrated local and segmental cord reflexes, or is to be found in the ultimate cellular reactions is not determined by the studies here reported.

CONCLUSIONS

1. Transection of the spinal cord in rabbits at the level of the 6th and 7th cervical vertebrae renders the animal completely poikilothermous over a period of at least 11 days. Experiments have not been extended to longer periods.

2. Following the taking of food, the largest temperature rise observed was 3.5°C. The maximum rise is found to occur in 7 to 12 hours after feeding.

3. Intravenous injections of vaccine in 4 animals from 2 to 4 days after cervical cord transection did not cause a rise in temperature. Such injections were quite toxic, led to a definite fall in temperature and early death.

4. Subcutaneous and intramuscular injections of vaccine in rabbits from 3 to 7 days after cervical cord transection cause a rise in temperature. The onset of this temperature began as a rule in 1 to 2 hours and varied between 1.2° and 2.9°, and could be initiated from a rather wide range of levels (36.1° to 39.4°). The high constant temperature level was reached in from 3 to 7 hours, and it persisted over a period of 12 hours.

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THE PHYSIOLOGICAL MAXIMUM OF BLOOD PRESSURE IN THE CAT

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On the basis of the view of the nature of the organism developed by Pike (1) that it is a mechanism in which the velocity of the reaction is dependent, in part at least, upon the volume of blood flowing through a given structure or organ, the question of blood pressure becomes one of the fundamental conditions in the regulation and maintenance of the activities of the organism. Since the rise of blood pressure following restriction or curtailment of the volume of blood flowing through the medulla may be regarded as an adaptation in accordance with the theorem of Le Chatelier, it is of some interest to determine whether the animal's response to the most severe change in conditions—the total deprivation of blood and oxygen—to which the central nervous system may be subjected, approaches the maximum response of which it is capable.

Stewart and his collaborators (2) in 1906 presented the picture of the typical anemic rise of blood pressure on occlusion of the arteries to the head. It has seemed worth while to find out how closely this may approach the maximum response to emergency on the part of the organism. In addition to the central station in the medulla, there are other points on the motor pathway which may be acted upon to produce more or less vasoconstriction. These are the myo-neural junction and the smooth muscle of the blood vessels.

The object of this series of experiments was therefore to determine how closely the anemic rise of blood pressure approaches the maximal rise of which the organism is capable, and at what point and by what agent acting on the motor pathway the maximum response of blood pressure could be elicited. In studying these responses, the following agents were used on

1. The central station in the medulla: Bulbar anemia, induced by occlusion of the head arteries.

2. The myo-neural junction of the blood vessels: Adrenalin, 5 cc. 1:10,000.

3. The smooth muscle of the blood vessels: Ergot, 1 cc. fluid extract. Pituitrin, ½ cc. Parke, Davis. Barium chloride, 5 to 10 cc. M/100 solution.

The usual technique for preparation of the head arteries (2) was followed on etherized cats, and a control occlusion and release were made. Blood pressure was measured by a mercury manometer. After recovery from this occlusion, a second was done. At the height of the cardiovascular response to this, the animal was injected in the femoral vein with one of the drugs under consideration to see whether any further rise in blood pressure could be brought about by added vasoconstriction, induced at the myo-neural junction or in the muscle itself, or whether during the anemic rise, the medullary effect is the maximum.

In all cases, the results were conclusive, as shown by the following table of blood pressures. (Table 1.)

It will be seen, therefore, that adrenalin causes no further rise in blood pressure beyond that induced by bulbar anemia. In 1922 Mrs. Winkin (3) showed that the curve of the anemic rise under repeated occlusion of the head arteries becomes dissociated into two distinct parts after several successive occlusions. Such a dissociation curve was obtained during the fifth occlusion of the above experiment.

TABLE 1

OCCLUSION NUMBER	DRUG INJECTED	MAXIMUM PRESSURE	REMARKS
1		200	
2		220	
3	Adrenalin	220	
4	Adrenalin	210	
5		220	"Dissociation curve"

The same process was repeated with drugs acting directly upon smooth muscle. From 2 to 5 cc. of an M/100 to M/25 solution of barium chloride in Ringer's solution were injected intravenously. In no case was there a greater rise in pressure than in the control occlusion of the head arteries, even if the amount of the drug injected was sufficient to cause the death of the cat. This is shown in the experiment of August 4th, when 2 cc. of M/25 $BaCl_2$ were injected. (Fig. 1.)

In several similar experiments, $\frac{1}{2}$ cc. of pituitrin in Ringer's solution was injected. While this caused a perceptibly extensive contraction of the abdominal smooth muscle, the rise in blood pressure was never to so great a height as the control. Fluid extract of ergot was likewise used, but caused only a relatively slight rise.

It is obviously necessary, however, for the medulla to be functionally active in order to produce this maximum pressor effect by means of bulbar anemia. In animals in which there has been failure of the medulla so that one had a spinal cat, injection of adrenalin or the smooth muscle stimulants was, of course, still effective. But it caused a rise of pressure

only to the height which was earlier attained during bulbar anemia, and never exceeded it, as the following tracing (fig. 2) and table show. (Table 2.)

Injection of those drugs which act directly upon smooth muscle does not produce quite so great a rise in blood pressure as adrenalin, which falls into line with what one would expect of the relative effectiveness of muscle and muscle and nerve.

The next step was to reverse the order of procedure and at the height of such a rise of blood pressure as might be induced by one of the foregoing drugs, to occlude the head arteries and produce bulbar anemia. This was done in a series of experiments (fig. 3).

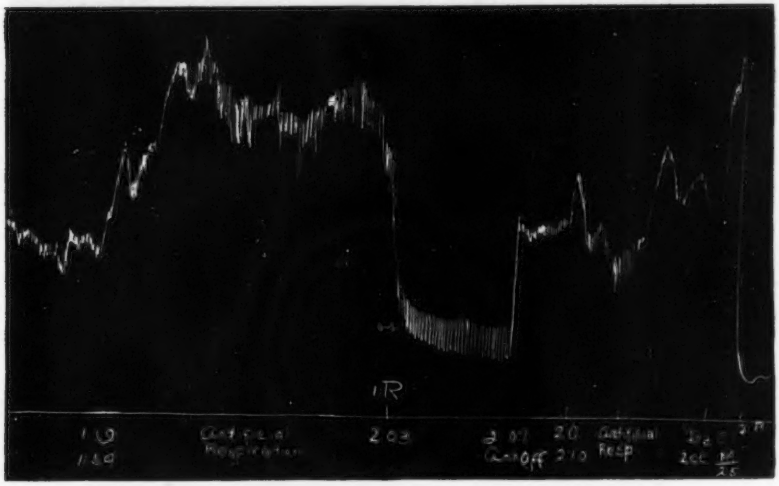

Fig. 1. The base-line represents zero blood-pressure. *O* indicates the time at which the head arteries were occluded, and *R* indicates the time of their release. Of the two anemic responses shown, the one at the left is the control, while that at the right shows the effect of the injection of barium chloride. Note that the maximum height which blood pressure attains is slightly greater in the control.

Following is a table typical of such an experiment. (See table 3.)

In some cases, however, adrenalin may raise the pressure to as high as 190 or 200 mm. Hg. In such cases, the increment of pressure induced by bulbar anemia is much less, varying between 40 to 20 mm. Hg. The nearer the physiological limit for the particular animal has been reached by the injection of adrenalin, the less the increment from bulbar anemia.

The injection of ergot causes a relatively smaller rise in pressure than the injection of adrenalin, and hence a relatively superposed anemic rise.

It would appear, then, that there is a physiological limit to the height to which blood pressure can rise, and this limit seems to be approximated

more completely by the stimulation of bulbar anemia than in any other manner. Such stimulation apparently involves a greater number of vasoconstrictor fibers than adrenalin affects, since after the injection of adrenalin, a further rise in pressure is obtained on occlusion of the head arteries, while the injection of adrenalin following occlusion of the head arteries appears to elicit no further rise in pressure, possibly because the involvement of vasoconstrictor fibers is already more nearly maximum.

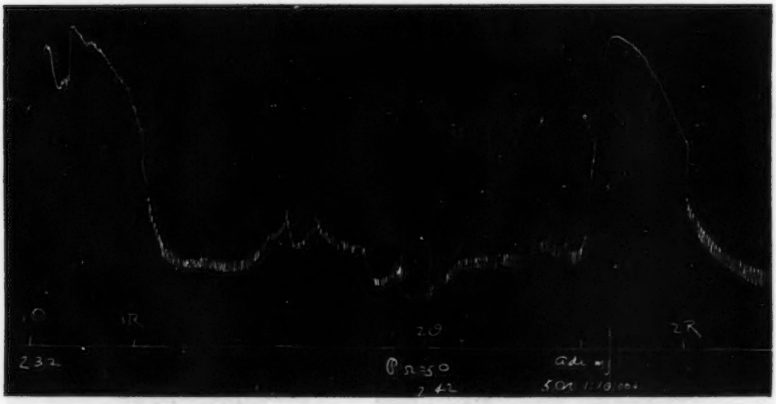

Fig. 2. The base-line represents zero blood-pressure. *O* indicates the time at which the head arteries were occluded, and *R* indicates the time of their release. The tracing at the left is the control. Pressure fell to spinal level, following it, and subsequent occlusions failed to elicit a response. After two minutes of the second occlusion, adrenalin was injected.

TABLE 2

OCCLUSION NUMBER	DRUG INJECTED	MAXIMUM PRESSURE	REMARKS
1		220	After the first occlusion, pressure
2 (ineffective)	Adrenalin	215	fell to the spinal level and the
3 (ineffective)	Adrenalin	220	other occlusions were ineffective
4 (ineffective)	Adrenalin	215	

Hooker in 1907 (4) advanced the conception of the physiological maximum of the heart rate, and Tulgan (5) has recently confirmed this on vagotomized cats, showing that under such circumstances the heart attains a maximum rate which is not altered by the injection of adrenalin. It has seemed to me possible to extend this conception of a physiological maximum to blood pressure as well as heart rate. There appears to be a fairly definite limit beyond which blood pressure does not rise.

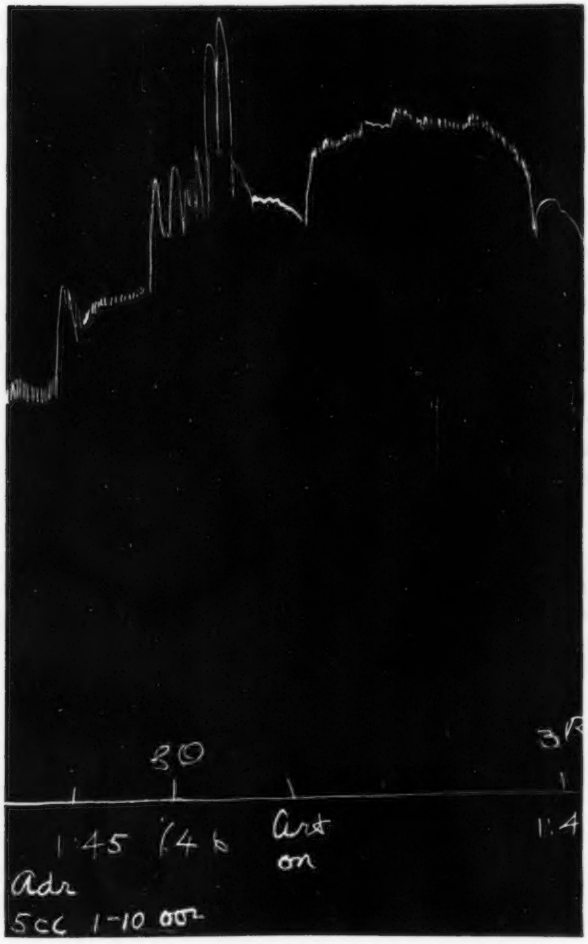

Fig. 3. The base-line represents zero blood-pressure. Reading from left to right, at 1:45 adrenalin was injected; *3 O* indicates the time at which the head arteries were occluded; *art. on*, when artificial respiration was applied, and *3R* when the head arteries were released from occlusion.

TABLE 3

CONTROL PRESSURE	MAXIMUM ADRENALIN PRESSURE	MAXIMUM OCCLUSION PRESSURE
100	145	220
105		200
95	140	180

On such a basis the high blood pressure, sometimes running above 300 mm. of mercury induced in such pathological conditions as nephritis, remains unexplained. The question may be raised as to whether it is to be interpreted as a response on the part of the organism to get more blood through the kidneys, whether there is some action on the smooth muscle directly, or whether there is some central condition involved.

SUMMARY

A number of experiments were performed on cats in which bulbar anemia was induced, to see whether the maximum rise in blood pressure is obtained by stimulation of the medulla or some point on the motor pathway. The results demonstrate that the height to which blood pressure rises during bulbar anemia is not exceeded when agents acting peripherally on the cardio-vascular system are employed.

I wish to make grateful acknowledgment to Prof. F. H. Pike of Columbia University for his suggestions and discussion of this work.

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THE RELATION OF THE PYRAMIDAL TRACT TO SPINAL SHOCK

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STATEMENT OF PROBLEM. Spinal shock has been defined as the transient depression of reflex activity following transection of the central nervous system. The evidence for the view that the principal factor involved is the loss of impulses traversing the descending tracts (1) is too familiar to require citation in detail. Yet one crucial experiment of Sherrington's may be recalled for it involves a principle upon which the present experiments depend. If, after recovery from spinal shock, a second transection be performed caudal to the level of the first, there is no return of shock. Sherrington also found that in the cat that has undergone preliminary decerebration followed by spinal transection, the flexion reflex shows no shock though the extensor reflexes are markedly depressed (2). Interpreting this result as a corollary of the first experiment, it may be argued that the decerebration severed descending tracts concerned in the maintenance of flexor tone, leaving intact the reflex arcs that dominate the extensors. Such an interpretation accords with the flexor atony and extensor rigidity following decerebration. During the interval between decerebration and spinal transection, recovery of the flexion reflex has progressed upon a purely spinal basis, and is unmasked when released by the subsequent spinal transection from the inhibition due to the reflex extension. A different view has recently been expressed by Forbes in the following paragraph quoted from his article in Physiological Reviews (3):

> The question of spinal shock is one upon which there is great disagreement among different authors. Originally Sherrington stated that spinal transection was followed by depression of all reflexes in the regions posterior to the transection, including flexor and extensor reflexes, but that the flexion reflex suffered much less and recovered more quickly than the extensor reflexes. More recently Sherrington and Sowton have reported that the flexion reflex in response to single shocks is actually increased by spinal transection, its threshold being lower and the contraction with a given stimulus being greater. In some experiments soon to be published, we have found the increase in the flexion reflex produced by single shocks to occur immediately after spinal transection. Sherrington originally mentioned the after-discharge as a feature of the flexion reflex which was especially impaired after spinal transection. I have seen the flexion reflex in a decerebrate cat changed by low spinal

transection from a small contraction with notable after-discharge to a brisk contraction with apparently no more after-discharge than appears in the twitch of an isolated muscle. Before transection, repeated stimuli caused cumulative contraction in a way that they failed to do after transection. It is quite possible, therefore, that the earlier statement about the flexion reflex taking part in the general depression known as spinal shock was due to the fact that it was then customary to use repeated stimuli to evoke all reflexes, and that therefore the cumulative effect resulting from after-discharge before transection led to a larger total contraction with this kind of stimulation than was found when the cumulative effect was abolished by transection. There is no doubt that in this reflex in the mammal the response to single stimuli is increased by spinal transection. The so-called shock effect is in this case not a depression but a modification of the reflex response.

Sherrington first called attention to the absence of shock to the flexion reflex after spinal transection in the decerebrate cat five years prior to his paper with Sowton (4) on single break shocks at a time, when he was using only faradic and mechanical stimuli (2). Consequently, the apparent discrepancy between results of transection in the intact and in the decerebrate cat cannot be solely due to more effectual summation in the presence of marked after-discharge. A more important factor present in both the experiments of Sherrington and Sowton and those of Forbes, Cobb and Cattell (5), is an interval between decerebration and spinal transection sufficient for a considerable degree of recovery from the shock due to cutting cerebral flexion arcs.

The present experiments were designed as a step toward the analysis of the cerebral arcs concerned in the reënforcement of the flexion reflex. In view of the ease with which flexion may be elicited from a relatively wide area of motor cortex and the relative rarity of extensor responses, the pyramidal tract might be expected to play such a rôle. If it does so then unilateral ablation of the motor area prior to spinal transection, should result in reduction of shock to the contralateral flexion reflex.

METHODS OF RECORDING. In employing such a method, it is essential to avoid extraneous causes of asymmetry such as might result from injury incident to dissection. Even the use of a stigmatic electrode is open to the objection that the needle may be imbedded more deeply in one foot than the other. Therefore recourse has been had to a simple yet fairly constant stimulus, the pinch of a bull-dog clamp. An isotonic method of recording has been used in which movement of the writing point is identical in range with movement of the foot. An 8-gram weight suffices to swing the lever to the limit of its excursion.

In the later experiments, after it had become evident that there was a constant alteration of the patellar jerk, this reflex was elicited by the pendular fall of a hammer through an arc of about 60°, the thigh being held vertical by two curved rods.

In large dogs, prolonged lying upon one side on a hard floor sometimes results in transient depression of both flexion and extension reflexes on that side. This effect has not been observed in cats or monkeys which have been kept on sawdust. Care has been taken to keep the animals in a symmetrical posture from the time of transection to the time of the first series of records. Thereafter the posture has been reversed at regular intervals. Thus if a dog lay the first night upon the right side, he lay the second upon the left.

After recovery of a moderate grade of flexor tone a constant weight was applied to secure initial extension when recording flexion reflexes. Even in large dogs this was never more than 95 grams.

RESULTS. *Controls:* Of five control cats in which spinal transection was the only operative procedure, four recovered spinal reflexes at relatively equal rate and to an equal degree upon the two sides. One cat showed marked asymmetry.

Ablation experiments: In 4 cats and 6 dogs the motor area of the cortex was ablated on the left. After an interval of 1 to 130 days the cord was transected.

Flexion reflex: In all 4 cats and all but one dog, the flexion reflex recovered more rapidly on the right than on the left. The degree of asymmetry was variable and showed no clear relation to the length of the interval between cortical ablation and spinal transection. It attained a maximum within one to six hours after transection. At twenty-four hours it was usually demonstrable though diminished by the relative recovery of the reflex on the left. In dogs spinal shock is more severe than in cats and the asymmetry more persistent. A slight trace of asymmetry may persist for weeks. A typical course of recovery of flexion in the dog is shown in the following protocol. Obviously the observed latent period represents a period of summation of subminimal stimulation.

Dog 1. (See figs. 2 and 5.) December 27, 1923.

Ablation of left sigmoid gyrus. Ether anesthesia. Considerable bleeding from dura and moderate amount from brain. Two hours after operation right hemiparesis, marked.

Can scarcely walk and tends to circle to left .

December 29th. Hemiparesis less intense but easily noticeable in both fore and hind limb. No spasticity at any time.

January 2, 1924. Ether anesthesia. Laminectomy and transection of cord in mid-thoracic region.

Operation begun at 11:04, completed at 12:08. Cord transected at 11:37.

Dog 1. 1/2/24, transection at 11:37.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	LATENT PERIOD IN SECONDS	DURATION OF STIMULUS IN SECONDS	TIME OF RECORD
Flexion reflex	Left digit 2	6.0	20.8	20.3	4:52
	Right digit 2	44.5	5.0	12.0	4:54
	Left digit 3	1.0	18.7?	30.0	4:57
	Right digit 3	37.0	0.4	7.3	4:59
	Left digit 4	11.0	4.3	22.5	5:02
	Right digit 4	30.0	9.3	25.5	5:05
	Left mid tarsus mesial	0		22.7	5:07
	Right mid tarsus mesial	0		22.7	5:09
Patellar reflex	Left patellar	44.5			
		69.0			
		67.5			5:11
	Right patellar	39.5			
		38.7			
		36.5			5:12

Dog 1. January 3, 1924. Lay on right side since yesterday. Leg held in extension by 95 grams weight.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	LATENT PERIOD IN SECONDS	DURATION OF STIMULATION IN SECONDS	TIME OF RECORD
Flexion reflex	Left digit 1	0		21.5	2:31
	Right digit 1	88.5	1.0	12.7	2:35
	Left digit 3	85.0	0.7	14.5	2:39
	Right digit 3	109.5	1.0	14.8	2:42
	Left mid tarsus internal	0		22.8	2:47
	Right mid tarsus internal	4.4	18.4	31.3	2:50
	Left digit 2	7.6	2.3	30.5	2:54
	Right digit 2	18.0	1.8	24.0	2:57
	Left digit 4	0		43.7	3:00
	Right digit 4	0		41.0	3:04
	Left digit 4	0		43.0	3:07
	Right digit 4	0		54.5	3:11
Patellar reflex	Right patellar	74.5			3:17
		84.0			
		80.0			
	Left patellar	39.0			3:20
		13.0			
		39.0			

Dog 1. January 4, 1924. After lying on left side. Extending weight of 95 grams.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	LATENT PERIOD IN SECONDS	DURATION OF STIMULATION IN SECONDS	TIME OF RECORD
Flexion reflex	Left digit 3	45.0	2.0	17.3	5:05
	Right digit 3	107.5	1.0	6.5	5:09
	Left mid tarsus internal	0		26.0	5:15
	Right mid tarsus internal	5?	13.0	37.2	5:19
	Left digit 4	64.5	1.2	20.0	5:40
	Right digit 4	43.0	1.2	23.0	5:42
	Left digit 4	75.5	1.3	16.0	5:45
	Right digit 4	19.0	9.5	35.0	5:48*
	Left digit 4	0		32.7	5:50
	Right digit 3	43.5	3.8	19.5	5:53
	Left digit 3	51.0	20.0	41.0	5.55†
Patellar reflex	Left patellar	111.0			6:00
		108.5			
		118.5			
	Right patellar	51.0			6:01
		47.0			
		59.0			
Flexion reflex	Left digit 3	108.0	2.7	9.7	6:06
	Right digit 3	12.5	15.0	34.0	6:08
	Left digit 3	54.0	17.0	35.7	6:10
	Right digit 3	86.0	3.1	27.0	6:13

* Dropped asleep.
† Woke.

Scratch reflex: Only 1 cat (cat 1) was transected at a sufficiently high level (7th cervical) to test the return of the scratch reflex. It recovered within a few hours upon the right but could never be elicited upon the left during the three remaining days of life.

Extension reflexes: The only unilateral extensor reflex to recover within the period of asymmetry is the patellar jerk. Of the 7 animals in which this reflex was recorded, 3 recovered it more rapidly on the left than on the right—the converse of the flexion reflex. In none was recovery notably quicker on the right. Dominance of the left patellar jerk is most definite in dog 2, figure 1. This result may be largely dependent upon the rela-

Dog 1. January 5, 1924. Has been on right side. Extending weight 95 grams.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	LATENT PERIOD IN SECONDS	DURATION OF STIMULATION IN SECONDS	TIME OF RECORD
	Left digit 3	0		27.7	1:49
	Right digit 3	92.0	1.7	11.0	1:55
Flexion reflex	Left digit 2	26.0	1.2	23.5	1:59
	Right digit 2	98.7	2.2	16.1	2:03
	Left digit 4	0		28.7	2:06
	Right digit 4	0?		40.8	2:10
	Left patellar	69.0			2:47
	Left patellar	88.7			
	Left patellar	85.0			
	Left patellar	96.5			
	Left patellar	105.0			
	Left patellar	106.0			
Patellar reflex	Right patellar	66.5			2:49
	Right patellar	41.0			
	Right patellar	49.5			
	Right patellar	34.0			
	Right patellar	40.0			
	Right patellar	34.0			
	Left digit 2	90.0	1.1	6.0	3:08
	Right digit 2	111.5	?	*	3:10
Flexion reflex	Right digit 2	0		28.0	3:13
	Left digit 2	64.7	1.8	9.5	3:15
	Right digit 2	95.5LL	1.1?	8.9	3:17

LL = Movement limited by limit of excursion of lever.
* Light touch.

tively greater flexion tonus upon the right. It is noteworthy that when the flexion reflex is increased, the excursion of the foot in the patellar reflex is frequently diminished and vice versa. The patellar jerk appears to be an exception to the rule of reciprocal innervation. In this reflex in man Golla and Hettwer find contraction of flexors begins within 7 to 10 sigma of onset of contraction of the extensors, acting as a check upon the excursion of the leg (6).

Monkeys: In 3 callothrix monkeys the leg area of the left precentral cortex was ablated after confirmation of localization by faradic stimula-

Dog 1. January 7, 1924. Extending weight 95 grams.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	LATENT PERIOD IN SECONDS	DURATION OF STIMULATION IN SECONDS	TIME OF RECORD
Flexion reflex	Left digit 3	6.5	?	32.0	12:25
	Right digit 3	40.5	1.2	12.5	12:28
	Left digit 3	0		32.0	12:32
	Right digit 3	0		51.3	12:36
	Left digit 2	16.0	1.1	30.7	12:40
	Right digit 2	3.7	4.6	40.6	12:44
	Left digit 2	50.0	1.3	18.4	12:49
	Right digit 2	63.5	2.9	18.0	12:53 *
	Left digit 4	0		31.0	12:56
	Right digit 4	0?		28.7	1:00
Patellar reflex	Left patellar	86.0			1:05
	Left patellar	83.3			
	Left patellar	78.4			
	Right patellar	73.0			1:08
	Right patellar	51.8			
	Right patellar	56.4			
	Right patellar	79.8			
	Right patellar	73.8			
	Right crossed patellar	3.2			1:10
	Right crossed patellar	7.0			
	Right crossed patellar	7.1			
	Left crossed patellar	27.5			1:12
	Left crossed patellar	38.5			
	Left crossed patellar	17.3			

* Weight removed.

tion. Spinal transection was performed in monkey 1 nineteen days later Sherrington states that "in the monkey in some instances three quarters of an hour or so after transection—a status supervenes in which with cold hands and ears the animal lies down listless, and perhaps unconscious with respiratory movements of the Chevne-Stokes type. This state may persist 12 hours or so and end either in gradual recovery or death" (7). Such was the state of monkey 1. He died 48 hours after transection. His reflexes could not be elicited by the constant stimulus employed in other cases. Shortly before death, however, there was a clear flexion response to pressure upon or stroking the lower abdomen. This reflex was far more vivid on the right than on the left, beginning on the right at the

Dog 1. January 8, 1924. Has lain on left since yesterday. Bed sore on right shoulder.

Patellar reflex investigated in greater detail.

First pendular after swing expressed in per cent of initial excursion.

The smaller percentage on the right is probably due to the greater flexor tone on the right which acts as a check upon both the initial excursion and the after swing.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	PER CENT SWING		DURATION OF STIMULUS IN SECONDS	TIME OF RECORD
Patellar reflex	Right patellar	99.4	54			3:00
	Right patellar	100.2	65	58		3:01
	Right patellar	104.0	54			3:02
	Left patellar	113.2	74			3:09
	Left patellar	114.5	59	64		3:10
	Left patellar	111.6	58			3:11
			LATENT PERIOD			
Flexion reflex	Left digit 3	34.0	10.0		24.0	3:31
	Right digit 3	83.0	0.5		7.5	3:33
	Left digit 4	6.5	3.0		26.3	3:37
	Right digit 4	18.0	9.4		24.0	3:40
	Left digit 2	11.0	2.0		29.4	3:44
	Right digit 2	87.5*	1.7		6.5	3:46
	Left digit 2	74.5	0.8		10.5	3:50
	Right digit 2	92.0*	1.3		8.5	3:54
	Left mid tarsus internal	0			32.7	3:57
	Right mid tarsus internal	0			26.3	4:01
	Left digit 1	3.5	8.2		29.0	4:05
	Right digit 1	49.0	10.2		23.2	4:08

* Movement limited by limit of excursion of lever.

1st or 2nd stroke, on the left only after 10 or more strokes. The movement was of far greater range and force on the right than on the left.

Monkey 2 was transected after an interval of 31 days. Though her general condition was excellent throughout, she suffered a prolonged period of shock. Reflexes did not recover to a degree adequate for recording for 35 days after transection. At no time did they show a significant degree of asymmetry.

Monkey 3 was transected after an interval of 10 days. Twenty-five days later there was sufficient reflex recovery for recording. At this time

Dog 1. January 9, 1924. Has continued lying on left owing to bed sore on right shoulder.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	LATENT PERIOD IN SECONDS	DURATION OF STIMULATION IN SECONDS	TIME OF RECORD
Patellar reflex	Left patellar	108.0			2:53
	Left patellar	100.5			3:01
	Left patellar	93.8			3:08
	Right patellar	109.6			3:22
	Right patellar	105.7			3:24
	Right patellar	110.0			3:26
Flexion reflex	Left digit 3	47.5	2.8	10.8	4:41
	Right digit 3	96.5	0.7	11.9	4:44
Crossed extension reflex	Right from left digit 3	0		17.0	4:46
	Left cross extension from right digit 3	15.5	1.5	14.3	4:49

the flexion reflex was stronger upon the right, the patellar reflex upon the left, just as in the dogs. There followed a period of reflex depression during which the reflexes could not be recorded. A week later they returned sufficiently for recording. Again the flexion reflex was stronger on the right. Though records were obtained for a considerable period thereafter, there was never again a significant right sided asymmetry.

RELATION OF DEGREE OF ASYMMETRY TO INTENSITY OF SPINAL SHOCK. Other things being equal, the degree of asymmetry is usually inversely proportional to the intensity of spinal shock. Thus monkey 1 recorded two days after transection gave an average flexion reflex on the right of 71.9 mm., on the left of 2.5 mm.; monkey 3, first recorded 25 days after transection gave a flexion reflex on the right of 65.3 mm., on the left of 26 mm.; monkey 2, first recorded 35 days after transection, gave an average flexion reflex on the right of 27.3, on the left of 39.5. Similarly dog 1 on the day of transection gave an average flexion reflex on the right of 37 mm., on the left of 6 mm.; while dog 3, which developed an infection from accidental opening of the frontal sinus in trephining, suffered a degree of spinal shock more intense than any of the other dogs and was the only dog to show no asymmetry.

RELATION OF DEGREE OF ASYMMETRY TO CEREBRAL DEVELOPMENT. It might be anticipated that the monkey with its highly developed cerebrum and wide range of skilled unilateral movement would show more marked asymmetry than the cat or dog. Where recovery from spinal

Dog 1. January 10, 1924. Patellar reflex investigated in greater detail as on January 8.

	POINT OF STIMULATION	MOVEMENT OF FOOT IN MILLIMETERS	PENDULAR AFTER SWING IN MILLIMETERS	PENDULAR AFTER SWING IN PER CENT OF INITIAL EXCURSION	TIME OF RECORD
Patellar reflex	Left patellar	121.0	92.4	76.0	2:48
	Left patellar	125.5	107.5	86.0	2:49
	Left patellar	121.5	99.0	81.0	2:50
	Right patellar	104.3	76.0	73.0	2:59
	Right patellar	110.5	69.0	63.0	3:00
	Right patellar	114.3	66.0	58.0	3:01
	Right patellar	111.3	30.0	27.0	3:05
			LATENT PERIOD IN SECONDS	DURATION OF STIMULATION IN SECONDS	
	Left extensor thrust digit 3 touch	31.0	0.6	4.0	3:13
Flexion reflex	Left digit 3	125.5	1.2	9.3	3:15
	Right digit 3	80.5*	1.7	11.2	3:18
	Right crossed extension from left digit 3	146.0	4.2	9.0	3:22
	Left crossed extension from right digit 3	42.0	1.0	29.0	3:25
Flexion reflex	Left mid tarsus internal	2.0	15.8	22.5	3:29
	Right mid tarsus internal	27.0	18.0	25.2	3:36
	Left mid tarsus internal	13.0	10.2	22.0	3:39
	Right digit 4	125.0*	1.0	8.5	3:44
	Left digit 4	96.0	1.4	12.2	3:48
	Left crossed extension from right digit 4	101.0*	0.8	4.5	3:52

* Movement limited by limit of excursion of lever.

shock is sufficiently rapid, this is true. Thus monkey 1 developed an asymmetry more marked than any other animal of the entire series.

ISOLATION DYSTROPHY. In the monkey, however, spinal shock is often so severe and prolonged as to merge with an increasing isolation dystrophy which may obliterate any tendency to asymmetry. So far as I am aware, up to the present time, Sherrington's theory of isolation dystrophy (8)

has rested solely upon physiological evidence. It seems therefore, highly significant that of the 7 spinal cords so far examined histologically that of monkey 2 which developed the most intense and prolonged spinal shock and showed no asymmetry, is the only one to show an abnormal condition

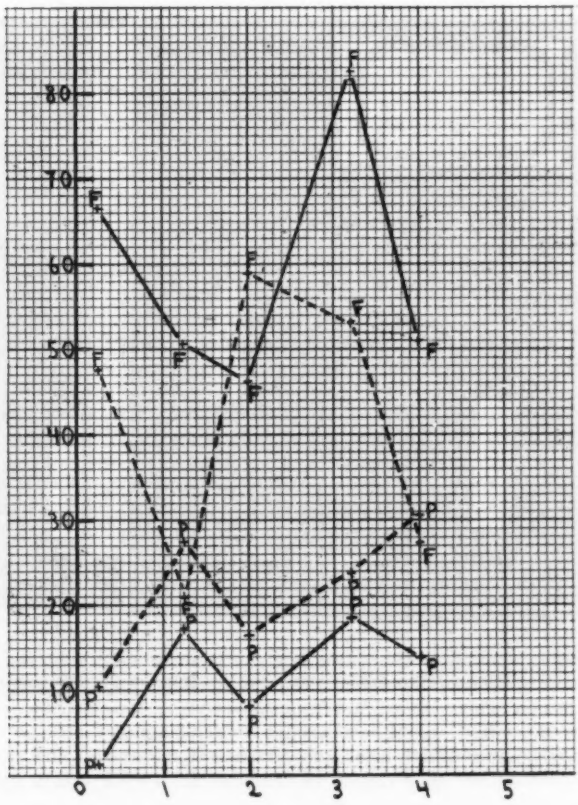

Fig. 1. Dog 2. Average of flexion reflex and of patellar reflex. Ordinate: movement of foot in millimeters. Abscissa: days after transection. Solid line: right. Broken line: left. *F:* flexion reflex. *P:* patellar reflex.

of the anterior horn cells of the lumbar segments, though those of the cervical region are normal. In the lumbar region the change is less severe in the lateral than in the mesial cell group. In the lateral group, the nuclei are displaced laterally, but the tigroid bodies appear normal. In

the mesial group, there is advanced chromatolysis, vagueness and sometimes loss of cell outline, and an excess of satellite cells.

RELATION OF ASYMMETRY TO EXTENT OF ABLATION. The degree and duration of asymmetry is proportional to the completeness of ablation of the motor area. Thus dog 1 in which the ablation includes almost the

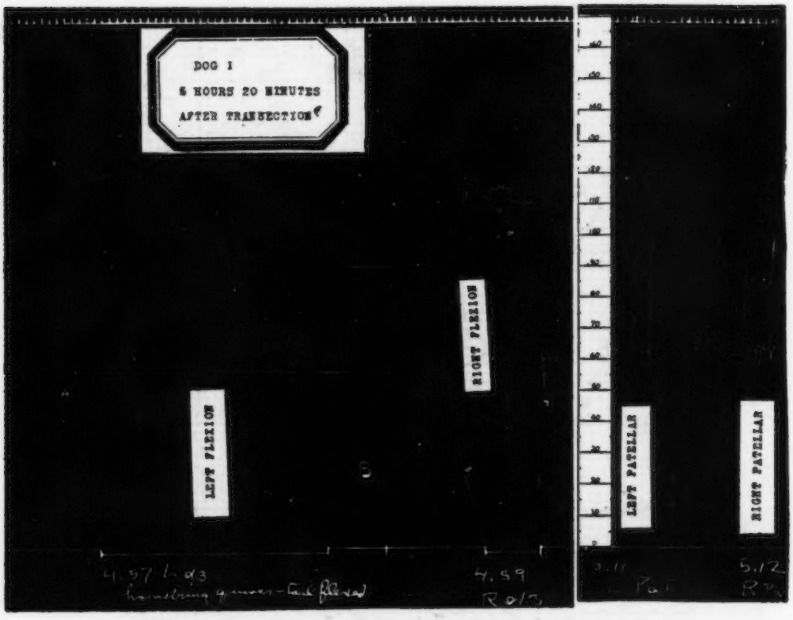

Fig. 2. Dog 1. Five hours and 20 minutes after transection. Time signal above in seconds set on arc of recording lever. Stimulus signal below set on arc of recording lever. Left flexion reflex. *A:* artifact from slight passive movement in application of stimulus to third digit. Right flexion reflex elicited 2 minutes later. Stimulus to third digit. *B*: Point at which lever was detached from left foot and attached to right. Rise in base line shows grade of flexion maintained by right leg prior to stimulation.

Left patellar reflex. Right patellar reflex.

entire left sigmoid gyrus, on the day of transection gave an average flexion reflex on the right of 37 mm., on the left of only 6 mm. and was almost as markedly asymmetric the following day; while dog 4, in which the lesion involved only the posterior mesial quarter of the gyrus, on the day of transection gave an average flexion reflex on the right of 57.4 mm., on the left of 36.2 mm., and on the following day the reflex was actually

slightly greater on the left. In comparing the results obtained from the monkeys with those from the dogs and cats, it may be significant that in most of the latter the ablation of the sigmoid gyrus was relatively complete; in the monkeys only that portion of the precentral gyrus was removed, to which the hind limb responded on faradization.

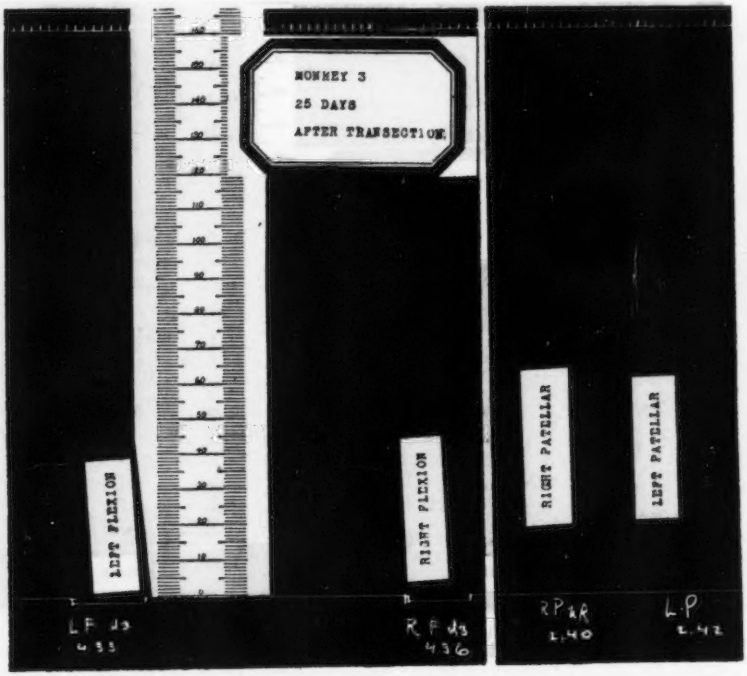

Fig. 3. Monkey 3. Twenty-five days after transection. Time signal above in seconds set on arc of recording lever. Stimulus signal below set on arc of recording lever. Left flexion reflex. Stimulus to third digit. Right flexion reflex elicited 3 minutes later. Stimulus to third digit. Right patellar reflex. Note dominance of flexors over extensors. Left patellar reflex 2 minutes later.

THE BASAL GANGLIA: An interesting comparison is afforded by 2 cats (cats 2 and 3) of the same weight and general condition with the same level of transection at the same interval after cerebral ablation, in one of which the left motor cortex was removed, in the other the entire left hemisphere. The degree of asymmetry is similar, but the degree of shock

presents a striking contrast. The weak stimulus used in recording induced a flexion reflex in the hemidecerebrate 54 minutes after transection. In the animal with ablation of only the motor area, the first response occurred 3 hours and 49 minutes after division of the cord. Such a result suggests that in the cat the basal ganglia are of far greater importance in reënforce-

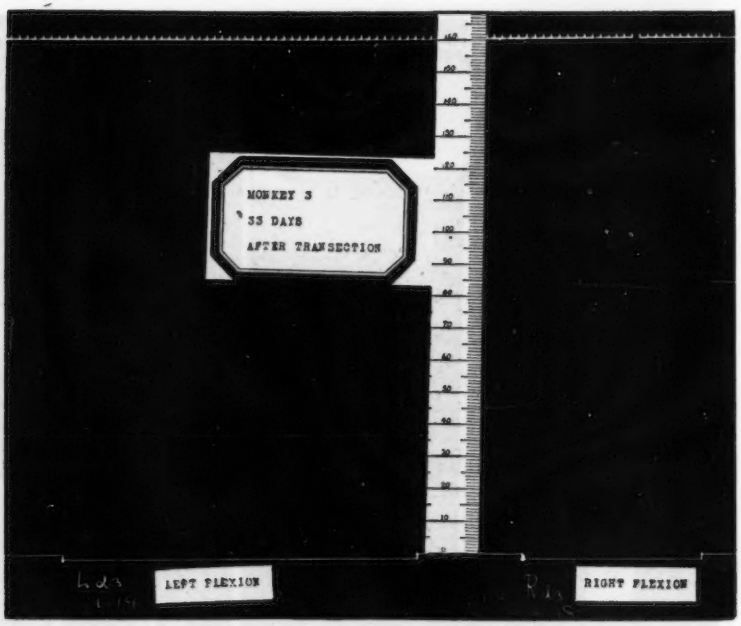

Fig. 4. Monkey 3. Thirty-three days after transection. Time signal above in seconds set on arc of recording lever. Stimulus signal below set on arc of recording lever. Left flexion reflex: stimulus to third digit. No response. Right flexion reflex elicited four minutes later. Stimulus to third digit. Note after discharge. Increase of flexion response during after discharge has occurred not infrequently in this series of experiments. These tracings suggest an algebraic summation of an ipsilateral extension reflex with brief after discharge and a flexion reflex with longer after discharge, such as have been described recently by Liddell and Sherrington in decerebrate preparations (9).

ment of the flexion reflex than is the motor cortex, though the bilateral distribution of their descending tracts prevents an increased asymmetry following their ablation. Obviously such a result needs repeated confirmation before conclusions may be drawn from it. These results are summarized in table 1.

TABLE 1

NUMBER OF ANIMALS*	INTERVAL IN DAYS BETWEEN CEREBRAL ABLATION AND SPINAL TRANSECTION	LEVEL OF TRANSECTION	AVERAGE MOVEMENT OF FOOT IN MILLIMETERS								ROUGH ESTIMATE OF PER CENT OF LEFT MOTOR AREA ABLATED
			Day of transection				1 day after transection				
			Flexion reflex		Patellar reflex		Flexion reflex		Patellar reflex		
			Right	Left	Right	Left	Right	Left	Right	Left	
											per cent
Cat 1	106	7 cervical	98.5	51.8			38.2	80.3			50
Cat 2	1	1 lumbar	27.6	3.7	14.5	13.4					100
Cat 3 (hemi-decerebrate)	1	1 lumbar	56.6	26.0							100
Cat 4	2	6 thoracic	19.1	4.8			47.3	44.4			70
Cat 5†	1	Midthoracic									15
Dog 1	6	Mid thoracic	37.0	6.0	38.3	90.3	55.1	23.1	79.5	30.3	80
Dog 2	24	Mid thoracic	66.7	47.7	1.3	10.7	50.6	21.0	17.2	27.5	70
Dog 3	6	Mid thoracic	29.3	41.0	35.3	28.5	15.0	48.5	24.0	46.3	60
Dog 4		Mid thoracic	57.4	36.2	19.8	20.5	41.6	69.1	69.7	52.0	25
Dog 5	130	Mid thoracic	33.0	5.5	41.4	26.0	51.5	0	60.0	56.1	80
Dog 6	44	7 to 8 cervical					74.9	19.0			85
		Day after transection first records obtained									
Monkey 1	19	11 thoracic	2 days after transection				71.9	2.5			22
Monkey 2	31	9 thoracic	35 days after transection				27.3	39.5			18
Monkey 3	10	1 lumbar	25 days after transection				65.3	26.0	0	17.0	25

* Numbering based upon order of mention in text, not upon the sequence in which experiments were performed.

† Records of cat 5 omitted because misleading, due to neglect to eliminate initial flexion posture (greater on right) when recording flexion reflex.

CONCLUSIONS

1. The loss of cortico-spinal impulses is a definite though relatively minor factor in spinal shock to the flexion reflex.

2. In the monkey, when spinal shock is sufficiently prolonged and severe, isolation dystrophy may lead to degenerative changes in the anterior horn cells below the level of transection.

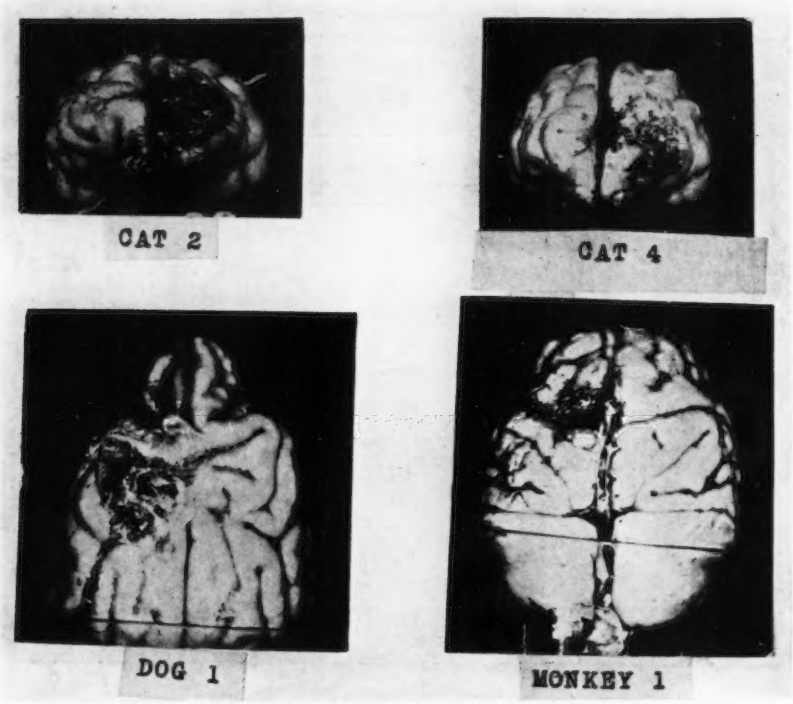

Fig. 5. Brains: Cat 2, cat 4, dog 1, monkey 1.

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Note: Only after this paper had been sent for publication have I read Sherrington's Marshall Hall Prize Address on the spinal animal delivered in 1899 (10), in which the following passage occurs:

> The cat from which the Rolandic area of the cortex has been removed, so as to ablate the whole of the limb centres from one hemisphere, if some weeks later "decerebrate rigidity be induced in its limbs, yields the rigidity without perceptible difference between the sides both right and left. But in the monkey similarly prepared, a great difference between the limbs of the two sides is apparent. The rigidity on the side crossed to the cerebral lesion is very much less than on the homonymous side.

CONDITIONS OF ACTIVITY IN ENDOCRINE GLANDS

XIV. The Effects of Muscle Metabolites on Adrenal Secretion

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In 1895 Johansson reported that the tetanizing of muscles increases the frequency of the pulse (1). He argued that the effect could not be reflex from sensory stimulation, on the following grounds: *a*, the latent period was too long (half a minute instead of a few seconds); *b*, the acceleration was greater the longer the tetanization; *c*, the stimulus was applied beyond a section of the spinal cord; *d*, exclusion of the blood stream from the tetanized muscles resulted in no or much reduced acceleration; and *e*, permitting the blood to flow through previously tetanized muscles caused temporarily a faster rate. Johansson drew the inference, therefore, that in muscular activity a substance is formed which accelerates the pulse. By isolating the heart from the central nervous system, and tetanizing the muscles of the hind portion of the body, also isolated, he again found that the cardiac contractions were accelerated. He concluded that the metabolites from laboring muscles increase the pulse frequency by direct action on the heart. Johansson's observations were made on dogs. Shortly after his paper appeared Hering reported that a change from rest to activity was attended by a faster pulse in the rabbit with a completely denervated heart, and suggested that the effect might properly be explained in Johansson's terms (2). Athanasiu and Carvallo likewise noted cardiac acceleration in dogs, anesthetized with chloralose, when they stimulated the posterior portion of the cut spinal cord (3); but apparently they did not denervate the heart, for they cite Johansson and Hering as having proved that toxic substances from muscular contraction act directly, since "one can completely denervate that organ without its losing its reaction to the chemical products of work."

The foregoing experiments have been rather widely accepted as showing that fatigue products exert a direct action on cardiac muscle, and thus evoke the residual increase of the pulse rate observed during muscular work when all extrinsic cardiac nerves have been cut (see Tigerstedt, e.g., 4). Under normal conditions it has been supposed that these products prob-

ably add their influence to other influences causing the faster pulse of muscular effort. Mansfeld, however, found that extracts of fatigued muscle produced no faster beat than extracts of normal muscle when injected intravenously, nor did the blood of an animal subjected to tetanization indicate that a circulating substance is the effective agent (5). The most abundant metabolites of muscular action are carbon dioxide and lactic acid. When carbon dioxide is added to the perfusing fluid, as shown by Ketcham, King and Hooker, it *depresses* the activity of the *isolated* heart, both of the terrapin and the cat (6). Peterson and Gasser were able to extract from fatigued muscle a substance or substances that affected the amplitude of the beat of *excised* hearts but had no influence on the rate (7). The conclusion that muscle metabolites exert direct accelerating action on the cardiac mechanism has, therefore, been called in question.

If chemical substances set free in the blood stream during vigorous work do not explain the faster beat of the denervated heart, how may the phenonenon be accounted for? Mansfeld argued that it was due to increased temperature, for *a*, no acceleration occurred in his experiments if the temperature of the right heart remained constant or increased only slightly; *b*, the faster beat accompanying muscular activity was first such as can be produced by injection of warm salt solution; and *c*, as inferred, afferent vagal fibers in the heart, sensitive to temperature changes, mediate a reflex speeding up of the rate by way of the accelerator center (5). Gasser and Meek, however, were unable to accept Mansfeld's inference of reflex acceleration due to increased temperature, for they observed as good effects after removal of the stellate ganglia (and thereby interruption of the efferent path) as before; and furthermore they found, in accord with Johansson and others, that exercise caused the pulse to become more frequent after all extrinsic nerves were cut (8). This effect, if due to rise of temperature, must be caused by direct action. The temperature did not rise in their experiments more than 0.5°C. Such an increase, according to effects on the isolated heart, would not explain the amount of acceleration which they observed. They suggest, therefore, that a higher temperature of the blood may be somewhat more effective on the heart in the intact animal than on the heart removed from the body. This granted, they would account for the residual increase, after denervating the heart, by the rise of temperature due to muscular activity, and not by the effects of metabolites.

Another factor may be at work which in our opinion has not been adequately considered by previous observers. That is the increase of circulating adrenin. Gasser and Meek did, indeed, notice that exercise a few hours after denervating the heart was accompanied by marked increase of the rate (64 and 68 beats per minute)—an effect which, by tying the "blood vessels to the adrenal glands," they reduced to 8 beats per minute. The reduction they attributed to "removal" of the adrenals (the residual

increase may have been due to escape of adrenin from the adrenals or to stimulation of the hepatic nerves (9)). They dismissed the adrenal factor as unimportant, however, because on the day after the operation the denervated heart did not accelerate 64 and 68 beats per minute, but only 18 and 12. They emphasize, however, that the larger effects were associated with a marked asphyxia, even to cyanosis, whereas the slighter effects, observed later, were accompanied by very few asphyxial symptoms. The absence of asphyxial symptoms in the later tests might have been due to the brevity of the exercise (30 seconds); in any case the effects were obtained under conditions quite different from those in Johansson's experiments and fail to account for the more rapid rate noted by him when muscle metabolites were set free in the blood stream.

The discrepancy between the positive effects of muscle metabolites on the denervated heart in intact animals, noted by Johansson and by Hering, and the negative effects on the extirpated heart, noted by Hooker and by Gasser and their co-workers, led us to make experiments which might be explanatory. Repeated use of the denervated heart has given an insight into the fairly prompt acceleration of its rate which occurs when material from the adrenal medulla (10) or from the liver (9) is given off into the circulating blood. Might not the acceleration seen by Johansson and by Hering be due wholly or largely to endocrine factors, and if so, do muscle metabolites as such have any positive influence on the beat? Our experiments were directed towards answering these questions.

The method. Johansson used dogs in his experiments. Hering used rabbits, and we have used cats. The animals were first anesthetized with ether, but usually at an early stage chloralose (3 cc. of a 1 per cent solution per kilo) was injected intravenously, and thereafter the ether was discontinued or was applied only as needed to prevent reflex movements during the completion of the operative procedures.

The heart was denervated by section of both vagi in the neck and removal of both stellate ganglia through the first intercostal space on each side. This part of the operation rarely required more than 15 minutes. The heart rate was recorded in a blood-pressure record taken from one of the carotid arteries.

Johansson noted that the muscle metabolites caused a marked increase in the amount of respiration. It seemed to us possible that the agent or agents influencing the respiration center might affect other medullary centers, and that records of expiratory volume might, therefore, be significant. In many of our experiments the minute volume of expired air was registered. A tube about 20 cm. long (to replace the natural dead space) was attached to a tracheal cannula; the other end of the tube was attached to a small chamber with two valves so arranged as to permit fresh air to enter but to cause the expired air to pass into a measured spirometer.

The muscle masses of the hind limbs were stimulated to activity either by applying electrodes to the lower portion of the severed lumbar cord, or the peripheral ends of both cut sciatic nerves, which were exposed near their emergence from the pelvis. When the sciatic nerves were excited Sherrington shielded electrodes were employed, in order to prevent spread of the current to neighboring tissues and thus to avoid stimulation of afferent nerve fibers. In some experiments involving sciatic stimulation, the lumbar cord was also sectioned in order further to assure absence of reflex disturbances. These different procedures caused no differences in our results. The muscles were made active by a tetanizing current sufficiently intense to produce strong contractions; a revolving wheel, with metal and ebonite sectors, that was placed in the primary circuit, interrupted the tetanizing current every other second. In each test the stimulation continued for five minutes. As the contractions weakened the secondary coil was usually advanced to maintain, so far as possible, the original vigor of response.

The record of blood pressure was taken to show *a*, the initial heart rate before stimulation was started; *b*, the rate during the first fifteen seconds of stimulation; and *c*, the rate at the end of each minute of stimulation. When the volume of expired air was observed, the change in the spirometer bell was noted at the end of each minute.

The rectal temperature was usually recorded minute by minute during the stimulation, but as the rise of temperature rarely exceeded 0.2°C., it was of minor importance.

In order to avoid exhaustion of the muscles by repeated stimulations, only one or two tests were made before excluding the adrenal glands. They were carefully excluded *via* the dorsal approach either by completely tying them off (by two ligatures slipped under the middle of each gland and the ligatures then tied around the opposite poles), or by complete extirpation, or by severance of the left splanchnic nerves and removal of the right adrenal. The method of adrenal exclusion had no effect on the results.

Results: In table 1 the data furnished by a typical experiment are presented, and in figure 1 are reproduced the initial records of the heart rate in that experiment and the records at the end of two minutes of stimulation, both before and after inactivation of the adrenal glands. In table 2 are given the maximal changes of rate of the denervated heart before adrenal exclusion in 13 of our 17 cases, and also, for comparison, the changes after the exclusion. Likewise, where observed, the expiratory volumes are reported during rest and during work and both with and without adrenals present. The results observed after the adrenals were inactivated are expressed in italicized figures. Of the four cases not detailed in table 2, one had a very rapid heart rate to start with (254 beats per minute) and stimulation of the separated part of the cut cord made the rate faster by

only 2 beats per minute; two showed a rise of 5 and 6 beats per minute that was followed by a fall below the original level; and the fourth was similar to these two in having in the test an increase of 2 beats the first minute followed by a decrease of 6 beats, and in another test a straight decrease of 8 beats. It is noteworthy that in the last instance the decrease ceased when the adrenals were inactivated. These last three anomalous cases will be considered later.

TABLE 1

Experiment 13 of table 2. Record of changes in the rate of the denervated heart, in the minute volume of expiration and in the temperature, during muscular contractions induced by stimulation of the peripheral ends of the cut sciatic nerves, before and after exclusion of the adrenal glands

Cat, male, ether followed by chloralose

	INITIAL	AFTER STIMULATING					
		15 seconds	1 minute	2 minutes	3 minutes	4 minutes	5 minutes
10:40							
Heart rate (per min.)	198	202	210	214	212	212	212
Temperature (°C.)		38.1	38.1	38.15	38.15	38.17	38.2
Volume expiration (cc.)	902		1464	1744	1704	1704	1704

Right splanchnics cut, left adrenal removed

11:53							
Heart rate (per min.)	184	184	184	184	186	186	186
Volume expiration (cc.)	862		1043	1143	1383	1444	1504
12:16							
Heart rate (per min.)	184	184	186	184	186	184	186
Temperature (°C.)	38.1	38.1	38.1	38.1	38.1	38.1	38.1
Volume expiration (cc.)	1003		1303	1243	1223	1404	1484
12:41							
Heart rate (per min.)	184	184	186	185	186	187	187
Temperature (°C.)	38.1	38.1	38.1	38.1	38.1	38.1	38.1
Volume expiration (cc.)			1163	1143	1303	1183	1203

Examination of table 2 reveals that in the 13 cases there summarized consistent results were obtained. Muscular activity, whether initiated by stimulation of the sciatic nerves or through an isolated part of the spinal cord, was accompanied by an acceleration of the denervated heart varying in all but two instances between 10 and 22 beats per minute. These observations are direct confirmation of the results noted by Johansson and by Hering. In 7 of these 13 cases the adrenal influence was excluded and the muscular stimulation was then repeated; in every instance

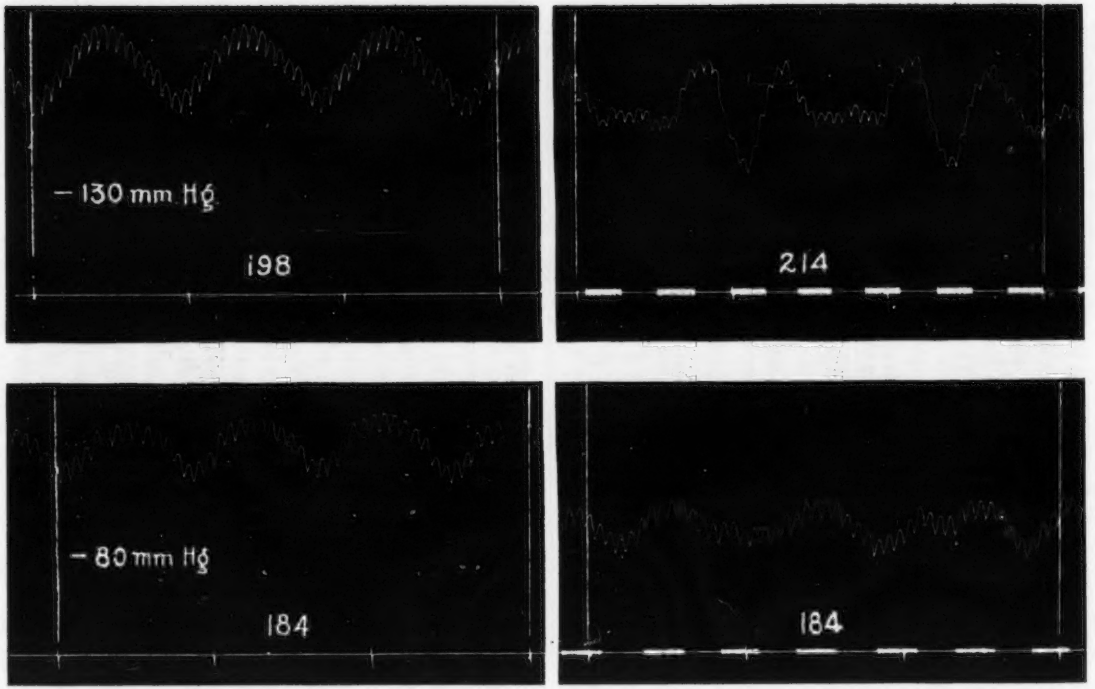

Fig. 1. Original records of rate of denervated heart. Upper record (10:40); initial rate 198 beats per minute; after 2 minutes of stimulation of peripheral ends of cut sciatics, 214 beats per minute. Base line marks 15 second intervals and 100 mm. mercury. Lower record (11:53), after right splanchnics cut and left adrenal removed; initial rate, 184 beats per minute; after 2 minutes of stimulation of peripheral ends of cut sciatics, no change. Base line marks 5 second intervals and 50 mm. mercury.

TABLE 2

Maximal changes in the rate of the denervated heart (increases unless otherwise indicated), and volumes of expired air, in a series of experiments in which the muscles of the hind legs were tetanized intermittently for 5 minutes

The figures in *italics* represent conditions after exclusion of adrenal activity.

EXPERIMENT	INITIAL HEART RATE	CHANGE IN HEART RATE PER MINUTE						AVERAGE BLOOD PRESSURE (mm. Hg)	5 MINUTE EXPIRATION VOLUMES		REMARKS
		15 seconds	1 minute	2 minutes	3 minutes	4 minutes	5 minutes		Rest	Stimulation	
									cc.	cc.	
1	157		−1	7	12	15					First experiment not complete; stimulation peripheral end of cut sciatics unless otherwise stated
2	196	1	6	10	16	16	14				Adrenals not excluded, volume expiration not recorded
3	168	0	0	0	2	16	20				Ether only, adrenals not excluded; volume expiration not recorded
4	178	1	4	7	8	8	9				Adrenals not excluded; cord transected
5	184	−2	3	5	6	4	4		4050	5634	Adrenals not excluded; lumbar cord transected
6	236	2	14	14	15	15	14		4752	8461	Adrenals not excluded
7	193	1	3	10	7	9	11	110			Ether only; volume expiration not recorded
	174	*2*	*2*	*4*	*2*	*4*	*4*	*100*			
8	206	2	6	12	12	12	10	130			Volume expiration not recorded
	162	*0*	*1*	*2*	*1*	*2*		*95*			
9	236	0	4	10	6	8	11	110	4270	5153	Stimulation of peripheral end of cut lumbar cord
	214	*0*	*1*	*0*	*1*	*0*	*−2*	97	*3670*	*4010*	
10	228	0	16	14	14	10	12	127	4432	7098	Same conditions as in experiment 9
	209	*0*	*1*	*1*	*−1*	*−2*	*−1*	*82*	*4792*	*6556*	
11	188	2	2	3	6	8	10	92	6436	7819	Same condition as in 9; considerable hemorrhage
	170	*−1*	*−3*	*−2*	*−6*	*−6*		*75*			
12	184	3	13	16	20	22	18	130	3388	7840	Cord cut in midlumbar region, but stimulation of cut sciatics
	170	*1*	*2*	*1*	*1*	*0*	*1*	*108*	3248	4852	
13	198	4	12	16	14	14	14	130	3810	6616	Volume expiration for only 4 minutes of stimulation
	184	*0*	*0*	*0*	*2*	*2*	*2*	*75*	*3784*	*5013*	

the typical increase in heart rate failed to appear. In one case after adrenal exclusion an increase of 4 beats per minute occurred during the five minutes of stimulation, but usually there was a variation up or down by only 1 or 2 beats. As shown in both table 1 and table 2 the volume of expired air increased markedly as a result of the muscular activity. In some of the experiments varying increases in heart rate indicated a correlation with varying increases in expiratory volume (see, e.g., expt. 13, table 1), but this proved to be not a general rule. All that we can state is that when the adrenal glands were intact muscular contractions caused a much greater ventilation of the lungs and a considerably faster beat of the denervated heart than prevailed before, and that when the adrenal glands were inactivated similar contractions for the same length of time again caused an increase in pulmonary ventilation, though not so great as previously, and no noteworthy or consistent increase in the heart rate.

Discussion. In the experiments above described precautions were taken against stimulation of afferent nerves and consequent reflex stimulation of adrenal secretion (10). When the electrodes were applied to the peripheral portion of the cut spinal cord they were insulated from underlying structures by a sheet of thin rubber. When the electrodes were applied to the sciatic nerves, spread of current was prevented by glass shields, and possible afferent impulses from the contracted muscles were in some instances excluded by section of the lumbar cord. Furthermore, we have stimulated muscles continuously for 30 seconds, in order to see whether effects characteristic of stimulating an afferent nerve for that period could be produced. They did not appear. By all these conditions we ruled out reflex influences. The effects were produced, therefore, by an agent carried away from the active muscles in the blood stream.

The agent could not have been warmer blood for, as illustrated in table 1, the increment of temperature was too slight to account for the faster heart rate (11).

The only other known agency which could have been derived from the muscles are the metabolites resulting from contraction—presumably carbon dioxide and lactic acid. These metabolites would account for the greatly increased respiration, such as occurred, for example, in experiments 6 and 12, table 2.

Evidence has been adduced in an earlier paper of this series that adrenal secretion is controlled by a center in the anterior portion of the floor of the fourth ventricle, and that under experimental conditions the activity of the center can be increased or decreased (12). The results reported in the foregoing pages are in harmony with the assumption that the metabolites which excite the respiratory center to greater action also excite the center for adrenal secretion. This assumption is based on *a*, the well-established sensitiveness of the denervated heart to increases in the amount

of circulating adrenin (10); *b*, the acceleration of the heart when muscle metabolites are increased so long as the adrenal glands are intact; and *c*, absence of such acceleration after the adrenals are inactivated. The chemical changes in the blood due to muscular activity do not directly affect the adrenal medulla. If they did so, the rate should still change considerably when one of the glands was denervated but left in the body, but under those circumstances the heart was not made to beat faster. Furthermore, the metabolites from muscular contraction do not speed up the heart rate directly, as Johansson had surmised. The absence of noteworthy acceleration after exclusion of adrenal influences is proof for that inference. And it is quite in accord with the negative results of perfusing the isolated heart with fluids charged with carbon dioxide or muscle extracts, that have been referred to earlier (6), (7). It may be argued that the volumes of expired air do not show that the respiratory mechanism was as much stimulated after inactivation of the adrenals as before, and that, since the conditions, apart from absence of the adrenal apparatus, were not identical, no reliable inference can be drawn. In experiment 12, table 2, however, the increase in expiratory volume was about 50 per cent after adrenal removal, without any noteworthy change of heart rate, whereas in experiments 5, 9 and 11, increases of expiratory volume considerably less than that, when the adrenals were present, were associated with typical cardiac accelerations. Again, as shown in table 1, an increase of pulmonary ventilation from 862 cc. per minute to 1504 was accompanied by a faster heart rate by only 2 beats per minute after exclusion of adrenal influence, while before that an increase from 902 cc. to 1464 was accompanied by an acceleration of 12 beats. And finally, though there was considerable stimulation of the respiratory center in all cases after the adrenals were rendered inactive, there was no consistent and corresponding change in the pulse. We conclude, therefore, that our assumption is correct, i.e., that the metabolites given off by active muscles are capable of exciting increased adrenal secretion by stimulating the nervous control of that secretion.

Quite possibly, under conditions as yet undetermined, the metabolites from muscular contraction may have a depressant action on the center for adrenal secretion. In several tests we have noted a slowing of the heart during the first minute, and rarely during the second minute of tetanizing the muscles, and only thereafter the typical increase. The 3 anomalous cases, referred to on page 157 in which considerable decreases of heart rate occurred, would be accounted for if the metabolites might have at times a depressant rather than a stimulating effect.

In the experiments here reported the nerves to the liver were not severed. Absence of noteworthy acceleration of the denervated heart after one adrenal was excised and the other was denervated allows the inference to

be drawn that the hepatic factor, if present, played a very minor rôle, and that the main cause of the faster rate was augmented adrenal secretion.

It would be a matter of considerable interest if as a consequence of blood changes from muscular contractions the respiratory volume could be multiplied five- or six-fold, as in normal vigorous exercise. We thought that we could perhaps bring about this result by use of a decerebrate preparation, but in our three attempts the spontaneous activity of the preparation made our efforts futile. Until these large effects are induced, we believe, it will be impossible to know to how great a degree the adrenal glands may be stimulated by blood changes due to muscular action.

We do not wish anyone to assume that the adrenal medulla is brought into extra action during muscular exertion solely by the chemical alterations of the blood induced thereby. Not improbably adrenal secretion is increased by nervous agencies, as the heart is, when skeletal muscles are innervated from the cerebral cortex. Evidence for this mode of controlling the glands is, however, still lacking.

SUMMARY

Johansson's observation is confirmed that the denervated heart is accelerated when muscle metabolites are set free in the circulation.

The acceleration fails to occur if the adrenal glands are rendered inactive; the inference is drawn, therefore, that the effect is mediated by these glands.

Reasons are presented for concluding that the muscle metabolites have no direct effect on the heart rate, but act by stimulating the nervous control of adrenal secretion.

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THE RELATION BETWEEN THE STIMULATING EFFICIENCY OF INTERMITTENT LIGHT AND THE LENGTH OF THE LIGHT AND THE DARK PERIODS

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It has been demonstrated in the butterfly, Vanessa antiopa, that the stimulating efficiency of intermittent light at optimum flash-frequency is higher with a ratio between the length of the light and the dark periods of 1/3 or 1/1 than it is with a ratio of 3/1 (1). This supports the conclusions that there are in the nervous system or the photoreceptors alternate sensitive and refractory periods[1] and that the latter are longer than former (5).

In this paper we shall extend the observations made on Vanessa, using more variations in the ratio between the length of the light and the dark periods and more suitable material, Eristalis tenax, with the view of ascertaining more precisely the relation between the length of these periods and stimulating efficiency.

The methods employed were essentially like those described in a preceding paper (6). The main features in the apparatus used consisted of two 110 volt, 1000 watt, stereopticon, monoplane filament, tungsten lamps of known efficiency so placed and screened as to produce two horizontal beams of light which crossed at right angles in the field of observation and a sector-disk on a motor with a speed regulator in each beam (6, fig. 1). The sector-disk in one beam was in all experiments rotated at high speed, so as to produce a flash-frequency of at least 125 per second with a stimulating efficiency known to be equal to that of continuous light. The light in this beam is referred to as continuous. The sector-disk in the other beam was rotated at various rates[2] and the opening in

[1] Fröhlich (4) observed that the so-called "action current" in the cephalopod eye is periodic, there being from 20 to 100 pulsations per second depending upon the intensity of the light received, the higher the intensity the higher the rate of pulsation. This is in close agreement with the results of our observation on the relation between stimulating efficiency, luminous intensity and flash-frequency. It consequently strongly supports the conclusions reached.

[2] This disk was on a motor run on storage batteries and the speed was regulated by varying the resistance in the circuit. In this way it could be very easily and accurately regulated.

it was changed, so as to produce in each series of tests the flash-frequency and the ratio between the length of the light and the dark periods desired.

The observations were made as follows: The lamps, the sector-disks and the motors were adjusted so as to produce in both beams an illumination of 115 m. c., a flash-frequency of 125 per second, and a ratio between the length of the light and the dark periods of 1/15.

A sheet of jet black paper (25 × 24 cm.) was now placed at the intersection of the two beams on a horizontal platform slightly lower than the lower edge of the luminous filament in the lamps; after which the field of light where the beams crossed was outlined with a pencil indicating the direction of the rays in each, and a line drawn bisecting the angle between these beams. A fly which was known to orient accurately in a single beam of light and which was dark-adapted for from 20 to 30 minutes, was then selected and placed facing the light in one of the beams near the inner edge and about 5 mm. from the corner of the field farthest from the sources of light (6, fig. 1). Its path was recorded by following it with a black pencil long enough to make it possible to keep the hand above the beams so as not to affect the direction of movement by reflected light. After it had crossed the field it was allowed to walk onto the pencil, with which it was transferred to the other beam, after which another path was made and recorded as before. Thus the specimen selected was allowed to make a total of 6 paths beginning each one in the same relative place alternately in the two beams so as to neutralize the effect of entering the field from one side. On completion of these six trials the fly was placed in darkness, and the black sheet containing the records of the six trials removed and filed.

Under the same conditions and in precisely the same way records of six trials were now successively obtained for each of 9 other dark-adapted specimens, all of which were placed in darkness after the tests. The flash-frequency in the beam of intermittent light was now changed to 66 per second after which records of six more paths for each of the 10 flies used in the preceding tests were obtained, the order of testing the different individuals being the same in these tests as it was in the preceding. This process was repeated until series of six paths for each of the 10 individuals selected were obtained for flash-frequencies of 125, 66, 50, 40, 33, 25, 20, 14 and 10 per second.

The opening in the sector-disk in each beam was now changed so as to produce a ratio between the length of the light and the dark periods of 1/10, and the two lamps were adjusted so as to produce an illumination of 115 m. c. in each beam.[3] On the following day the ten specimens used

[3] It should be emphasized that the illumination was in all tests precisely the same in the two beams, and that it was in all tests approximately 115 m.c. It was measured by means of a Sharp-Millar photometer calibrated just before the experiments.

in the preceding tests, which had now been in darkness nearly 24 hours, were tested with intermittent light of this ratio and different flash-frequencies until a record of 6 trials for each specimen for each of the flash-frequencies in the series presented above were obtained. This was repeated with the same specimens on the following day with the ratio in the intermittent light 4/1 and again the following day with 7 of the 10 specimens and 3 fresh ones with the ratio 1/3, and finally with 10 fresh specimens with the ratio 1/1. Thus there were obtained records of 6 trials in each of the following flash-frequencies; 125, 66, 50, 40, 33, 25, 20, 14 and 10, for each of the following ratios between the length of the light and the dark periods: 1/15, 1/10, 1/3, 1/1 and 4/1, a total of 45 groups of records.

TABLE 1

Relation between optimum flash-frequency and relative length of light and dark periods in intermittent light of 115 m.c.

FLASH FREQUENCY PER SECOND	RELATION IN LENGTH OF PERIODS									
	Light 1	Dark 15	Light 1	Dark 10	Light 1	Dark 3	Light 1	Dark 1	Light 4	Dark 1
	Average angle of deflection		Average angle of deflection		Average angle of deflection		Average angle of deflection		Average angle of deflection	
125	0.167	c	0.082	i	1.149	c	2.334	c	2.65	c
66	1.601	c	4.177	i	1.133	i	3.149	c	1.20	c
50	2.065	c	5.833	i	2.182	i	1.683	c	2.68	c
40	1.415	i	12.535	i	5.116	i	0.115	c	2.21	c
33	9.827	i	10.265	i	9.098	i	2.579	i	1.79	c
25	**15.814**	i	**11.661**	i	**14.809**	i	9.798	i	3.54	c
20	**19.081**	i	**13.166**	i	**13.266**	i	**14.444**	i	4.14	c
14	7.093	i	10.704	i	4.966	i	**12.998**	i	**5.60**	c
10	3.765	i	2.782	i	1.316	i	10.698	i	4.01	c

The angle of deflection from the line bisecting the angle between the two beams of light was ascertained for each path and from these the average for each group was calculated. These averages are presented in table 1 and figure 1. Those in which the average deflection was toward the continuous light were labelled *c*, and those in which it was toward the intermittent light, *i*. Consequently in all paths with angles of deflection labelled *c*, the stimulating effect of the continuous was greater than that of intermittent light and in all labelled *i*, it was less.

By referring to this table and figure it will be clearly seen that as the flash-frequency decreases the stimulating efficiency of the intermittent light in all of the ratios except 4/1, increases rapidly from a condition in which it is equal to that of continuous light to a maximum which is much higher after which it decreases. It will also be seen that for the ratios between the length of the light and the dark periods, 1/15, 1/10 and 1/3

the flash-frequency for maximum stimulation is approximately the same, it being between 20 and 25 per second, probably around 22, but that for the ratio 1/1 it is considerably lower, i.e., about 16 per second, while for the ratio 4/1 there is no indication of a maximum whatever.

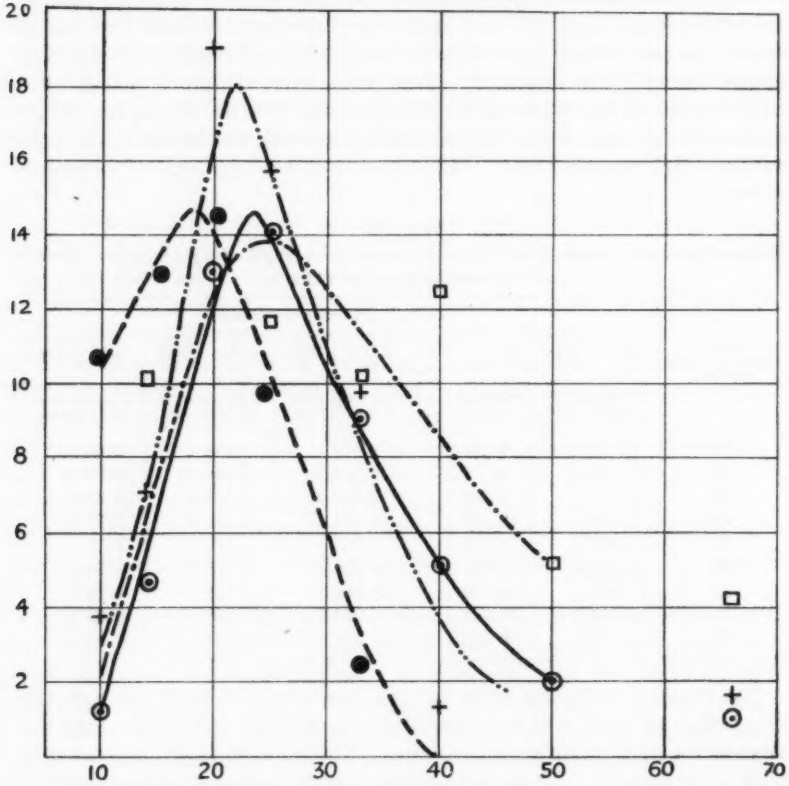

Fig. 1. Relation between stimulating efficiency of intermittent light and flash-frequency, with the following ratios between the length of the light and the dark periods; ●, 1/1; ⊙, 1/3; □, 1/10; +, 1/15; flash-frequency, abscissae; angle of deflection or reaction, ordinates.

These facts support the contention that in the process of orientation in insects in continuous illumination light does not act continuously, that there are in the nervous system or the photoreceptors alternate sensitive and refractory periods; and they throw considerable light on the length of these periods under various conditions, as the following discussion shows.

The results presented indicate, as previously stated, that in an illumination of 115 m.c. the flash-frequency for maximum stimulating efficiency is approximately 22 per second with the ratios between the length of the light and the dark periods 1/15, 1/10 and 1/3, and approximately 16 per second with the ratio 1/1. They also indicate that at the flash-frequency of 22 per second the stimulating efficiency is practically the same for the ratios 1/15, 1/10 and 1/3, but that it is considerably less for the ratio 1/1 (table 2). This difference in stimulating efficiency is probably due either to the difference in the length of the light periods or the length of the dark periods or both, since the amount of light-energy per flash is the same for all of the ratios. However, if the stimulating efficiency of the ratios 1/15, 1/10 and 1/3 with light periods 0.0028+, 0.0041+ and 0.0113+ is the same, as the results indicate (table 2), it is probable that the difference in

TABLE 2

The length of the light and the dark periods for maximum stimulating efficiency in intermittent light of 115 m.c.

FLASH FREQUENCY PER SECOND	RATIO BETWEEN LENGTH OF LIGHT AND DARK PERIODS	LIGHT PERIOD		DARK PERIOD	STIMULATING EFFICIENCY
		Second	Energy	Second	
22	1/15	0.0028+	x	0.0426+	Maximum
22	1/10	0.0041+	x	0.0413	Maximum
22	1/3	0.0113+	x	0.0340+	Maximum
22	1/1	0.0227+	x	0.0227+	High
22	4/1	0.0363	x	0.0090+	Very low
16	1/15	0.0039+	1.37+x	0.0585+	Low
16	1/10	0.0056+	1.37+x	0.0581+	High?
16	1/3	0.0156+	1.37+x	0.0468+	Low
16	1/1	0.0312+	1.37+x	0.0312+	Maximum
16	4/1	0.05	1.37+x	0.0125	Lowest

stimulating efficiency for these ratios and the ratio 1/1 is not due to difference in the length of the light periods but to difference in the length of the dark periods. If this is true and if the hypothesis previously formulated is valid, then the refractory period in an illumination of 115 m.c. is longer than the dark period for the ratio 1/1, or 0.0227+ second, and probably somewhat shorter than the dark period for the ratio 1/3 or 0.034+ second. It may then be concluded on the basis of the assumptions made that the length of the refractory period lies between 0.0227+ and 0.034+ second, probably very near the latter and that the sensitive periods vary in length inversely with the intensity of the illumination during these periods.

Reference to figure 2, in connection with the following paragraphs, will make plain these deductions. In this figure the relation between the light and the dark periods under various conditions is graphically illus-

trated, the blank spaces representing the light periods and the lines the dark periods. According to the hypothesis set forth the following obtains in continuous illumination: Beginning immediately after the organism is illuminated light acts for a short time inducing certain changes, after which it ceases to act and reverse changes take place, which may be thought of as processes of restitution; then, after these processes are complete and the system has assumed its former condition or nearly so,[4] light acts again for a time, after which restitution again occurs, etc., the intervals during which light acts being the sensitive periods, and those during which restitution occurs the refractory periods (5).

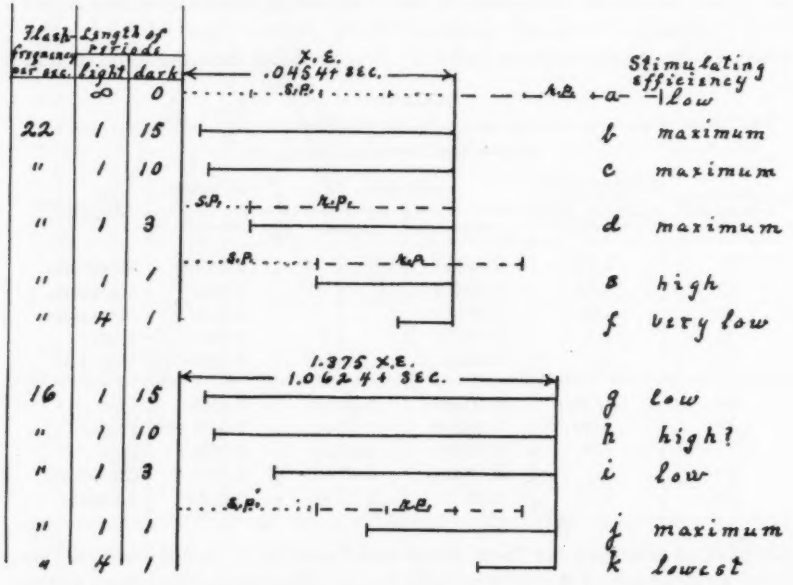

Fig. 2. Illustration representing the length of the light and the dark periods in relation to the sensitive and the refractory periods in intermittent and continuous illumination, 115 m.c. Blank spaces, light periods; lines, dark periods; *s.p.*, sensitive periods; *r.p.*, refractory periods; *x.e.*, given amount of light-energy.

This is illustrated in line *a*, in figure 2, in which *s. p.* represents the sensitive period during which *x* energy is received inducing some change, presumably photochemical in nature, and *r. p.* the refractory period, during which the system returns toward the condition which obtained at the beginning of the sensitive period, i.e., the beginning of exposure in

[4] The system probably does not return completely to its former condition, for if it did there would be no adaptation to light.

continuous illumination. If the light that is received during the refractory period has no effect and if the sensitive period, as indicated, is 4/3 as long as the refractory period, 3/7 of the light received has no effect and is wasted. Consequently the stimulating efficiency of intermittent light in which the dark periods correspond with *r. p.* and the light periods with *s. p.*, each delivering *x* energy, should be 7/4 as great as that of continuous light.

The conditions of maximum stimulating efficiency observed in 115 m.c. are represented in lines *b*, *c*, *d* and *j*. In *b*, *c*, *d*, *e* and *f* the light periods deliver *x* energy and the stimulating efficiency of *b*, *c* and *d* is maximum and approximately the same while that of *e* and *f* is lower. If we now assume that the effect of the light in the light periods is independent of the rate at which the energy is delivered the system would be in precisely the same condition at the end of the light periods in *b*, *c* and *d* as it is at the end of the sensitive period *s.p.* in continuous light. For maximum stimulating efficiency the dark period must be long enough to admit of complete restitution. If it is not restitution will continue on in the following light period resulting in waste of a certain amount of light. The dark period may, however, be longer than is required for restitution, since this would merely delay the beginning of the following sensitive period and would not result in the loss of light-energy. The fact that maximum stimulating efficiency is obtained under conditions represented in *b*, *c*, and *d* is consequently in harmony with the hypothesis presented, and if the stimulating efficiency under all of these conditions is actually maximum and equal it is plain that the refractory period can not be longer than the dark period in *d*, i.e., 0.340+ second. Under conditions, *e*, we find the stimulating efficiency lower than under conditions, *d*. If the stimulating efficiency is not dependent upon the rate at which the energy is delivered this difference can not be due to inequality in the length of the light periods under the two conditions, for the energy delivered per period is the same in both. It must therefore be due to the difference in the length of the dark periods. In accord with our hypothesis the more nearly the dark periods coincide with the refractory periods the higher the stimulating efficiency. If this is true, it follows from the facts presented that the dark period in *e*, is shorter than the refractory period, which signifies that it is not long enough for complete restitution. It may then be concluded, if the postulates made are valid, that the refractory period is longer than the dark period in *e*, namely, 0.0227+ second and not longer than the dark period in *d*, namely, 0.0340+ second.

On the basis of this conclusion the relatively very low stimulating efficiency under conditions, *f*, is intelligible. The same amount of energy per flash is delivered as under conditions *b*, *c*, *d* and *e* but the dark period is much shorter, being only 0.009+ second. If the refractory period is

approximately 0.034+ second it is evident that restitution will not be complete at the close of the dark period under conditions, *f*, and will extend over into the following light period resulting in a waste of light approaching that in continuous illumination, and the results presented in table 1 indicate that the stimulating efficiency is only a trifle higher than it is in continuous illumination.

It may now be asked if all this is valid, why with the ratio between the light and the dark periods 1/1, is the maximum stimulating efficiency at a flash-frequency of 16 per second in place of 22 per second, why is it that under these conditions the maximum stimulating efficiency appears to be practically as high as it is at a flash-frequency of 22 per second with the ratio between the length of the light and the dark periods 1/15, 1/10 and 1/3, and why is it that with the ratio 4/1 the stimulating efficiency is practically the same for all flash-frequencies?

By referring to table 2 it will be seen that at a flash-frequency of 16 per second there is delivered per flash about 1/3 more light-energy than at a flash-frequency of 22 per second. Consequently if the energy delivered per flash at a flash-frequency of 22 per second is sufficient to produce a stimulating effect, at a flash-frequency of 16 per second enough energy will be received to produce the same effect when the flash is about 3/4 complete and if the refractory period begins at this point, in accord with the hypothesis presented, the remaining energy, namely, about 1/4, will be wasted. This holds for all of the ratios. The maximum stimulating efficiency at a flash-frequency of 16 per second with the ratio 1/1, should, therefore, be somewhat lower than it is at one of 22 per second with ratios 1/15, 1/10 and 1/3. The results presented in table 1 indicate that it is somewhat lower than with the ratio 1/15 but not with the ratios 1/10 and 1/3. Now, it so happens that in obtaining the results with the ratios 1/10 and 1/3 specimens which had been in the laboratory for some time were used, whereas in obtaining those with the ratios 1/15 and 1/1 fresh specimens were used. Consequently only the results obtained under these two conditions are strictly comparable, and the fact that the maximum is lower under the latter than the former is in accord with our hypothesis. The difference in the condition of the specimens used may, therefore, account for the discrepancy in regard to the ratios 1/10 and 1/3 in comparison with the ratio 1/1.

The question as to why the maximum at flash-frequency 22 per second occurs with ratio 1/3, while at 16 per second it occurs with ratio 1/1 may be answered as follows: By referring to table 2 it will be seen that at a flash-frequency of 22 per second the shortest dark period for maximum stimulating efficiency is found with the ratio 1/3 and that it is 0.034+ second. This indicates, as previously demonstrated, that complete restitution requires approximately 0.034+ second. It will also be found that at a

flash-frequency of 16 per second the dark period with ratio 1/1 is 0.0312+ second, that is, that under these conditions the dark period is not long enough for complete restitution. However, if the light period is about 1/4 longer than necessary for stimulation, as previously shown, restitution will begin when this period is about 3/4 complete and continue during the rest of it (0.0078 second) and on during the dark period which follows. If we now add this portion of the light period (0.0078 second) to the dark period (0.0312+ second) we have 0.039+ second, which is ample time for complete restitution, if the conclusion reached regarding the time required for this process is correct. If this is true, the refractory period would not extend over into the following light period. At a flash-frequency of 16 per second we would, therefore, expect maximum stimulating efficiency with the ratio between the length of the light and the dark periods 1/1 but we would also expect the same efficiency with the ratios 1/3, 1/10 and 1/15. The results presented in table 2 indicate, however, that it is lower for all of these ratios. We are at present unable to explain this discrepancy.

Let us now consider the question concerning the absence of a maximum with the ratio 4/1.

The relation, under these conditions, between the light and dark periods and the sensitive and refractory periods is illustrated in table 3 and figure 3. The table shows that the dark periods at all of the flash-frequencies tested are much shorter than the refractory periods and that the energy delivered during a light period is not sufficient for stimulation except at flash-frequencies 20, 14 and 10 per second. The figure shows that this results in a distribution of the dark periods about equally among the sensitive and the refractory periods except at flash-frequency 14 per second in which the dark periods fall largely in the refractory periods. We would consequently expect a stimulating efficiency practically equal to that of continuous illumination except at a flash-frequency of 14 per second with which we would expect an efficiency somewhat higher but not nearly so high as that obtained with the ratio 1/3 at a flash-frequency of 22 per second. The fact that no increase in stimulating efficiency was observed at this flash-frequency probably indicates that the sensitive period is not strictly inversely proportional to the luminous intensity during the light periods and that the length of the refractory period depends upon the luminous intensity during the light period and during the refractory period.

The results obtained in a quantitative investigation concerning the magnitude of the difference between the stimulating efficiency of continuous and intermittent light, now under way, indicate that the refractory period is much longer when it occurs in light, as it does in continuous illumination, than it is when it occurs in darkness as it does in intermittent

light of the optimum flash-frequency and the optimum ratio between the length of the light and the dark periods, indicating that light retards restitution. Moreover, Folger (3) in observations on Amoeba found that the stimulation period, which is probably analogous to our sensitive period, is not inversely proportional to the luminous intensity, and that

TABLE 3

Relation between sensitive and refractory periods and light and dark periods with a ratio of 4/1

x represents the amount of light-energy per flash required for stimulation.

FLASH FREQUENCY PER SECOND	LIGHT PERIOD	INTENSITY DURING FLASH	ENERGY PER FLASH	DARK PERIOD	REFRACTORY PERIOD	AVERAGE ILLUMINATION
	second			*second*	*second*	*m.c.*
40	0.02	k	0.55 x	0.005	0.034	115
33	0.0242+	k	0.666+x	0.0060+	0.034	115
25	0.032	k	0.888+x	0.008	0.034	115
22	0.0363+	k	x	0.0090+	0.034	115
20	0.04	k	1.1 x	0.01	0.034	115
14	0.0571+	k	1.57 +x	0.0142+	0.034	115
10	0.08	k	2.2 x	0.02	0.034	115

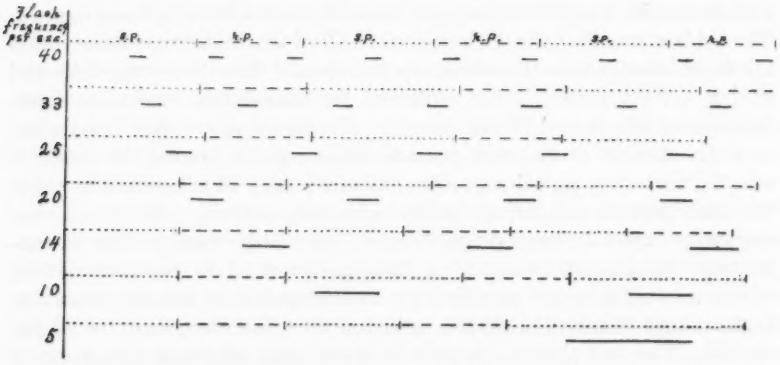

Fig. 3. Relation between light and dark periods and sensitive and refractory periods in intermittent light of 115 m.c. with the light periods 4 times as long as the dark periods. Blank spaces, light periods; lines, dark periods; dotted lines, sensitive periods; broken lines, refractory periods.

the latent period, which is in part similar to our refractory period varies with the intensity.

These facts all indicate that the conclusion reached in this and the preceding papers, regarding the length of the refractory period, applies only to intermittent light of the luminous intensity, flash-frequency, and ratio between the length of the light and the dark periods specified.

SUMMARY

1. In an illumination of 115 m.c. the stimulating efficiency of intermittent light with a ratio between the length of the light and the dark period 1/15, 1/10 or 1/3, and a flash-frequency of 50 per second, or higher, is practically equal to that of continuous illumination. As the flash-frequency decreases from this rate the stimulating efficiency increases to a maximum which is much higher at about 22 per second and then decreases until it is about equal to that of continuous light at 10 per second.

2. With the ratio between the length of the light and the dark periods 1/1 the flash-frequency for maximum stimulating efficiency is about 16 per second and with the ratio 4/1 no maximum was observed, the stimulating efficiency being practically equal to that of continuous illumination for all flash-frequencies tested.

3. The maximum stimulating efficiency is practically the same in magnitude for the ratios between the length of the light and the dark periods 1/15, 1/10 and 1/3 and probably somewhat lower for the ratio 1/1.

4. There probably are in the nervous system or the receptors alternate sensitive and refractory periods, the sensitive periods probably vary inversely and the refractory periods directly with luminous intensity.

5. In intermittent light with an average illumination of 115 m.c., a flash-frequency of 22 per second and a ratio between the length of the light and the dark periods of 1/3, the refractory period is approximately 0.034 second. In continuous illumination it is probably much longer indicating that light retards restitution.

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THE ACTION OF MINUTE AMOUNTS OF BARIUM CHLORIDE UPON THE KIDNEY

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Previous experiments from this laboratory (1) showed that when a rabbit's kidney is perfused with hirudinized blood at constant rate of flow per minute, the addition of adrenalin to the blood is followed by increased perfusion pressure, increased kidney volume and increased urine formation. These facts were interpreted to mean that adrenalin action includes constriction of vas efferens, thereby increasing glomerular pressure and distention.

Experiments planned to reveal similar action in eviscerated rabbits showed that in a few instances the injection of minute amounts of adrenalin or of pituitrin is followed by coincident increase in arterial pressure, increase in kidney volume, decrease in renal blood flow and increase in urine formation (2). This coincidence was regarded as showing that a constrictor substance acting upon the renal vessels in high dilution might produce more effective constriction of the efferent vessel than of the afferent. The former is usually the smaller vessel and if two vessels of different calibers are subjected to equal degree of constriction, greater increase in resistance must be produced in the smaller vessel.

It is the purpose of this note to make record of similar experiments with barium chloride, in which a similar coincidence was discovered.

Rabbits were used exclusively. The technique was identical with that previously described. It included administration of glucose before the experiment to make the animal diuretic and injection of 1 mgm. of atropin to lessen bronchial secretion. Other details were urethane narcosis supplemented by ether when necessary, tracheal cannula, record of arterial blood pressure from the carotid artery, excision of stomach and intestines after ligature of their arterial supply, ligation of right kidney, introduction of left kidney into an oncometer connected with a small Brodie bellows, cannula in left ureter connected with a drop recorder, section of the left splanchnic nerves and determination of blood flow through left kidney according to the method of Barcroft and Brodie, the abdominal aorta, lumbar veins and adreno-lumbar vein having been ligated. Injections of barium chloride in minimal amount of fluid were made by way of the jugular vein.

The record of one experiment is reproduced below. It shows that the injection of 0.5 mgm. $BaCl_2$ was followed by increase in number of drops of urine, swelling of kidney, rise in arterial blood pressure, and decrease in rate of blood flow through the kidney. Renal blood flow was estimated by measuring with a stop-watch the time required for 2 cc. of blood to flow from the renal vein into a graduated tube connected with the central stump of the inferior cava below the renal vein during temporary obstruction of the cava above the renal vein.

Nine experiments were made in which the technique was sufficiently perfect to justify consideration. A larger number of experiments was discarded because of obvious faults in technique or accidents during their course. The experiment reproduced is the most striking of the nine. In two others results of the same character were secured. It must be remembered that our aim was to operate within a range of dosage in which the constrictor action of the barium should be limited to the smallest arterioles, and it seems obvious that this range, assuming it to exist, must be small.

It may seem arbitrary to attach meaning to exceptional experiments of this character in which the effects are slight. Great care was taken to develop a technique which would permit the conclusion that results obtained were real. The value of this experiment consists in the support which it gives to the view that conditions favoring or retarding glomerular filtration may be created by alterations in the relative caliber of the efferent and afferent vessel, a view which is attractively reasonable, but extremely difficult to put to experimental test. While it is manifestly impossible to rule out other factors, such as changes in permeability and reabsorption, and in secretory activity if it exists, emphasis may be laid upon the fact that stimulation of smooth muscle is the most conspicuous feature in the

Fig. 1

action of barium in mammals; upon the fact that it has been shown to resemble Ca in diminishing permeability (3); and upon the fact that following parenteral injection in mammals, it is eliminated in the urine, either not at all, or in traces only (4). Its most easily demonstrable action on the kidney is contraction of vessels and suppression of urine. When, therefore, it is found that minute amounts still contract renal vessels as evidenced by diminution in blood flow, but increase kidney volume and urine, the explanation which appears most intelligible and to involve the introduction of the fewest unknown factors is that which we advance, viz., constriction of vasa efferentia.

There is an analogy between these experiments and those in which the renal vein is obstructed. It will be recalled that Heidenhain's ascription of "secretory" power to glomerular epithelium was partly based upon failure of obstruction of the renal vein to increase urine. But it has been shown (5) that conditions can be created of such a sort that compression of the renal vein does not decrease blood flow, and that then increase in venous pressure causes increase in urine. Increased glomerular pressure and decreased rate of blood flow through glomerular capillaries are antagonistic factors in the glomerular elimination of fluid. This antagonism is inevitably encountered in such experiments as that described in this paper, assuming that the reasoning on which the experiment was based is even approximately correct. It is another reason why a series of uniformly successful experiments is not to be expected.

Following Heidenhain, certain investigators, confronted by discrepancies between relations of urine formation to renal blood pressure and blood flow, have had recourse to "vital" theories of glomerular function. In more recent times, the discovery that urine flow and renal blood flow may vary quite independently, in the absence of changes in pressure sufficient to be explanatory, has suggested that changes in permeability of glomerular membranes may be important. In the absence of direct evidence on this subject, attention may properly be recalled to the possibility of internal adjustments of glomerular pressure of the sort defined in this paper.

SUMMARY

In exceptional experiments, minute amounts of barium chloride introduced into the circulation of an eviscerated rabbit caused increase in arterial pressure, in kidney volume and in urine flow, together with decrease in renal blood flow. Constriction of the efferent glomerular arteriole is regarded as the most probable explanation of this coincidence.

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A DESCRIPTION OF THE GLOMERULAR CIRCULATION IN THE FROG'S KIDNEY AND OBSERVATIONS CONCERNING THE ACTION OF ADRENALIN AND VARIOUS OTHER SUBSTANCES UPON IT[1]

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The results of experiments on the action of adrenalin on the perfused kidney (1) and on the influence of minute amounts of adrenalin and pituitrin on the kidney *in situ* (2) furnished the reason for beginning the observations recorded in this paper. Conditions were encountered in the rabbit's kidney which were regarded as evidence that a constriction of the efferent vessel caused by these substances might so influence pressure in the glomerular capillaries as to increase urine and cause swelling of kidney volume. It occurred to us that direct observation of glomerular vessels might be possible in the frog's kidney by methods of illumination and observation such as had been used by Krogh in his study of capillaries in muscles (3), and that it might thus be possible to measure the size of the glomerular tufts when subjected to influences similar to those studied in the rabbit's kidney. Preliminary experiments showed that the glomerular circulation in the frog's kidney may readily be made available to direct observation. A partial answer has been secured to the question stated,

[1] An account of the earlier experiments described in this paper was included in a lecture delivered before the Harvey Society on February 28, 1921 (Richards: Amer. Journ. Med. Sci., 1922, clxiii, 1; The Harvey Lectures, Series xvi, 1920–21, 163). The preparation here described was demonstrated before the Physiological Society of Philadelphia on January 17, 1921, before the American Physiological Society on December 29, 1921 (THIS JOURNAL, 1922, lix, 489) and before the Thirteenth International Congress of Physiology, Edinburgh, on July 22, 1923 (Quart. Journ. Exper. Physiol., Suppl. volume, December 1923, 235).

In October 1921 (Brit. Journ. Exper. Path., 1921, ii, 205) Hill and McQueen described experiments on glomerular capillary pressure in which direct observation of the frog's kidney was utilized. We do not know whether their experiments antedate ours or not.

The thesis of partial activity of the kidney which we presented in the reports mentioned and develop in greater detail in this paper has been accepted by Khanolkar (Journ. Path. and Bact., 1922, xxv, 414) and given further support by his indirect experiments on the mammalian kidney.

and in addition certain interesting features in the behavior of the glomerular circulation not hitherto described have been studied.

In the majority of experiments *Rana pipiens* was used; in some, *R. catesbiana* or *R. palustris*. The brain was destroyed by pithing or the animal was anesthetized with urethane.

The kidney was exposed by longitudinal abdominal incision. The anterior abdominal vein was cut between ligatures, its cut tributaries being cauterized. For intravenous injection, a glass cannula was tied into its central stump. The abdominal cavity was held open by pins thrust through the right parietal wall. In female frogs the ovaries and sometimes the right oviduct were dissected out, hemorrhage being prevented by ligature and cautery. In male frogs, in experiments in which observations of a large surface of the kidney were desired, the right testis and fat bodies were excised after ligature of their vessels.

Illumination of the kidney was best secured by means of a small arc-lamp. The rays were condensed to a circle of about 5 mm. diameter, and were cooled by passage through a layer of water tinged with methylene blue contained in a rectangular museum jar of 50 mm. thickness. The light was directed at the ventral surface of the kidney at an angle of 30 to 40 degrees.[2]

It was important that the ventral surface of the kidney should lie as nearly as possible in a horizontal plane. This was effected by placing small pledgets of cotton under the kidney after having divided the peritoneum at its lateral border. Surface reflections and drying were prevented by laying a small fragment of cover slip on the ventral surface.

Most of the observations here recorded were made with a monocular microscope, using the low power objective (16 mm.).

The area of the kidney surface found to be most suitable for study is the space between the adrenal body and the outer border. When first examined the vessels most clearly seen are the veins. Flow of blood in them is rapid or slow, constant or intermittent, depending on the condition of the animal and amount of blood lost during the preliminary operations.

The arterial circulation can be distinguished by the direction of its flow and by its greater rapidity. The tubules are commonly seen indistinctly. In the interstices of the veins one sees circular arrangements of capillaries which are obviously glomeruli. They measure from 140 to 300 microns in diameter. Movement of blood in them varies: in some it is of bewildering rapidity through a maze of pathways: in others, it is a slower progression of cells through fewer channels. The outline of the capsule can be made out: in some the glomerular tuft appears to fill the capsular

[2] In later experiments, made to confirm and amplify these, the method of illumination by transmitted light used by Wearn and Richards (p. 209) was adopted.

space: in others it does not, circulation being distinguishable only in parts of the area surrounded by the capsule. Figure 1 is a photomicrograph of a fairly typical field in the living kidney.[3]

In our earliest observations, using methods less perfect than those subsequently developed, the quite obvious differences in the appearance of neighboring capillary tufts caused a good deal of uncertainity. It seemed hardly possible that such great differences in blood flow could be encountered in glomeruli in the same kidney. Accordingly, an experiment was carried through in which five closely adjacent glomerular tufts of different appearances were studied in the living kidney, the animal killed and its kidney hardened *in situ* with formalin: thick sections were

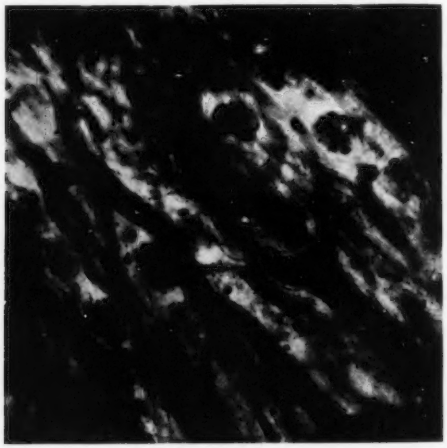

Fig. 1. Photomicrograph of the living frog's (R. pipiens) kidney (× 44) showing arterioles, veins and glomeruli.

cut, in one of which were found the five glomeruli observed during life. Identification was made certain by their arrangement, the distances between them, and their relation to two needle stabs marked with india ink made after fixing the kidney but before removing it for section. In the light of later experience this somewhat laborious effort was superfluous, but at the time it was useful in giving assurance that the character of blood flow through closely adjacent glomeruli might differ widely.

[3] The photomicrographs illustrating this paper were made with the assistance of Mr. R. C. Bradley to whom we express our thanks. All were made on living kidneys in which blood circulation was active. None of the negatives or prints have been retouched for reproduction.

VARIATIONS IN THE NUMBER OF "ACTIVE"[4] GLOMERULI. The number of active glomeruli to be found on inspection of the ventral surface of the kidney varied greatly in different animals, and also in the same animal under different conditions. In one animal, a single field of 2 mm. diameter may show as many as ten: in another, only two or three are to be found on searching the entire available surface of the kidney. The following observation is interesting, in that it was the beginning of a series of tests bearing upon this point.

December 15, 1920. R. pipiens, ♀, prepared as described. Considerable hemorrhage during dissection. Small pieces of very fine silk thread were laid transversely across the surface of the right kidney at intervals of approximately 2 mm., thus dividing the portion of the kidney to be examined into five convenient fields. The total number of visible glomeruli in the five fields was thirteen, of which five were active and eight inactive. The abdominal cavity was then filled with 0.65 per cent sodium chloride solution. After thirty minutes the glomerular count of the five fields was repeated. The total number of visible glomeruli was twenty-eight, all of which were active. Hence the absorption of salt solution into the circulation caused the resumption of blood flow in eight glomeruli in which circulation had ceased but which were visible because of stagnant corpuscles in their capillaries, and in fifteen which had previously been invisible.

This experiment was important in that it gave us our first numerical expression of the fact that under varying conditions of the renal circulation the blood circulates through varying numbers of glomerular tufts. The experiment was repeated a number of times with confirmatory results, and then the same procedure was used in testing a variety of agencies. These included intravenous injection of whole blood from another frog (plethora), intravenous injection of isotonic NaCl, of urea, glucose, sodium sulphate, caffeine, sodium bicarbonate, adrenalin and pituitrin, and section and stimulation of the rami communicantes which supply sympathetic fibers to the kidney. Typical results follow:

Plethora. 1. December 16, 1920. Rana pipiens. Pithed. Little hemorrhage. Glomeruli in 6 fields counted: active, 9; inactive, 2; total, 11.

The same immediately after intravenous injection of 0.5 cc. of hirudinized blood from another frog: active, 36; inactive, 1; total, 37.

The same after filling abdominal cavity with salt solution: active, 47; inactive, 0; total 47.

2. December 17, 1920. Very satisfactory preparation. Entire available kidney surface between adrenal and lateral border counted: active glomeruli, 10; inactive, 27; total 37.

Immediately after intravenous injection of 0.5 cc. of whole blood drawn from another frog: active, 30; inactive, 14; total, 44.

Five minutes later: active, 39; inactive, 7; total 46.

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[4] Throughout this paper the expression "active glomeruli" means glomeruli through whose capillaries blood cells can be seen to be moving.

Intravenous injection of isotonic sodium chloride solution. 1. December 21, 1920. R. catesbiana, ♀. Good preparation. Eight fields counted. Active glomeruli, 41; inactive, 9; total, 50.

Ten minutes after injection of 0.5 cc. 0.6 per cent NaCl: active, 54; inactive, 8; total, 62.

Thirty minutes after injection: active, 44; inactive, 6; total, 50.

2. December 22, 1920. R. pipiens ♀. Circulation sluggish. Six fields counted. Active glomeruli, 4; inactive, 9; total, 13.

Two minutes after intravenous injection of 0.05 cc. 0.6 per cent NaCl: active, 10; inactive, 5; total, 15.

Intravenous injection of urea. 1. December 21, 1920. R. Catesbiana. Eight fields counted.

Thirty minutes after injection of salt solution (see above): active glomeruli, 44; inactive, 6; total, 50.

Immediately after injection of 0.1 cc. 20 per cent urea solution: active, 65; inactive, 2; total, 67.

2. December 22, 1920. R. Pipiens. Seven fields counted. Active glomeruli, 32; inactive, 19; total, 51.

Immediately after injection of 0.05 cc. 20 per cent urea: active, 34; inactive, 13; total, 47.

Eight minutes later, 0.1 cc. 20 per cent urea was injected. Two minutes after injection: active glomeruli, 40; inactive, 6; total, 46.

Sixteen minutes after injection: active, 60; inactive, 7; total, 67.

Twenty-four minutes after injection: active, 43; inactive, 8; total, 51.

Intravenous injection of glucose. January 21, 1921. R. pipiens, ♀. Eight fields counted.

At 11:20.....Active, 31; inactive, 12; total, 43
" 11:35.....Injected 0.1 cc. of 10 per cent glucose solution
" 11:36.....Active, 47; inactive, 10; total, 57
" 11:50.....Active, 54; inactive, 7; total, 61
" 12:10.....Active, 62; inactive, 3; total, 65

Injection of sodium sulphate. 1. January 25, 1921, R. pipiens ♂. Considerable hemorrhage during preparation. Seven fields counted.

At 10:05.....Active, 6; inactive, 0; total, 6
" 10:08.....Injected 0.1 cc. 5 per cent Na_2SO_4
" 10:10.....Active, 35; inactive, 0; total, 35
" 10:25.....Active, 51; inactive, 0; total, 51
" 10:41.....Active, 48; inactive, 2; total, 50

2. January 25, 1921. R. pipiens, ♂. Prepared with little hemorrhage. Seven fields counted.

At 12:28.....Active, 28; inactive, 11; total, 39
" 12:31.....Injected 0.1 cc. 1.25 per cent Na_2SO_4
" 12:32.....Active, 34; inactive, 4; total, 38
" 1:33.....Active, 44; inactive, 5; total, 49

Injection of caffeine. January 27, 1921. R. pipiens, ♂. Little hemorrhage. Eight fields counted.

At 3:35.....Active, 84; inactive, 0; total, 84
" 3:39.....Injected 0.05 cc. 2 per cent caffeine
" 3:45.....Active, 99; inactive, 0; total, 99
" 4:15.....Active, 116; inactive, 0; total, 116
" 4:40.....Active, 103; inactive, 0; total, 103

Section of sympathetic nerve fibers to the kidney. April 25, 1924. *R. pipiens*, ♀. Right kidney prepared for examination by reflected light. Rami communicantes of 5th and 6th spinal nerves carefully dissected and a very fine silk thread laid beneath them. The nerves were protected with cotton moistened with Ringer's solution before and after section. Glomeruli were counted in five fields of kidney surface (only active glomeruli could be distinguished):

At 11:26–11:31.....12
" 11:31–11:35.....11
" 11:35–11:39.....10

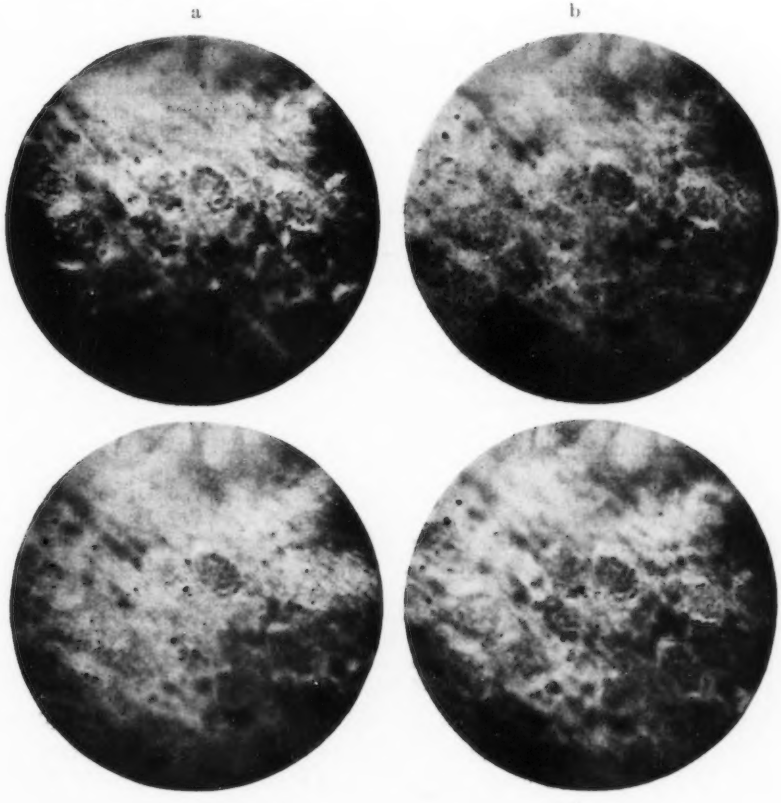


Fig. 2. Photomicrographs showing diminution in number of active glomeruli following the intravenous injection 0.2 cc. 1:20,000 adrenalin. Rana pipiens. Magnification = × 30. Exposures, ½ second. *a*, Control, 12 minutes before the injection of adrenalin; *b*, 87 seconds after adrenalin; *c*, 2 minutes, 25 seconds after adrenalin; *d*, 8 minutes after adrenalin. Focus unchanged throughout. In the original negatives, ten glomerular tufts can be identified in *a*, seven in *b*, 2 in *c*, and eight in *d*.

At 11:41 sympathetic rami of 5th and 6th nerves were cut
" 11:42–11:47.....15
" 11:48–11:53.....17
" 11:57–12:01.....17

Injection of adrenalin. December 23, 1920. R. catesbiana, ♀. Little hemorrhage. Three fields counted.

At 3:45.....Active, 37; inactive, 7; total, 44
" 3:55.....Injected 0.1 cc. 2 per cent caffeine
" 4:03.....Active, 48; inactive, 0; total, 48
" 4:40.....Active, 49; inactive, 1; total, 50
" 4:50.....Injected 0.1 cc. of adrenalin, 1:100,000
" 4:50.....Active, 12; inactive, ?; total, ?
" 4:57.....Active, 48; inactive, 0; total, 48

Injection of pituitrin. December 27, 1920. R. pipiens. Six fields counted.

At 2:40.....Active, 12; inactive, 3; total, 15
" 2:43.....Injected 0.05 cc. of Pituitrin "S", 1:1000
" 2:50.....Active, 22; inactive, 0; total, 22
" 3:03.....Active, 16; inactive, 2; total, 18
" 3:08.....Injected 0.05 cc. Pituitrin "S", 1:1000
" 3:10.....Active, 25; inactive, 1; total, 26
" 3:50.....Active, 14; inactive, 5; total, 19
" 3:54.....Injected 0.1 cc. Pituitrin "S", 1:1000
" 3:55.....Active, 0; inactive, 16; total, 16
" 4:00.....Active, 5; inactive, 12; total, 17

Stimulation of sympathetic fibers to the kidney. April 25, 1924. R. pipiens, ♀. Right kidney prepared for examination by transmitted light. Fifth spinal nerve root exposed, cut distal to ramus and ramus laid over fine platinum electrodes supported on the frog board. The nerve was protected by cotton moistened with Ringer's solution except during the period of stimulation. Glomeruli were counted in four rather large fields:

4:01–4:06.....4, 7, 6, 11: total, 28
4:06–4:10.....4, 7, 7, 11: total, 29
4:12–4:36.....Fifth nerve dissected and ramus placed on electrodes
4:38–4:40.....3, 10, 5, 10: total, 28
4:42–4:45.....3, 10, 5, 11: total, 29
4:46–4:48.....Ramus subjected to mild faradic stimulus
———.....0, 3 (i), 2 (1 i), 8 (1 i) ; total 10 active, 5 inactive
4:49–4:52.....4, 9, 3, 8 (1 i); total, 24 active, 1 inactive
4:54–4:56.....4, 9, 4, 9 (1 i); total, 26 active, 1 inactive

The four photomicrographs shown in figure 2 demonstrate the alteration in number of visible glomeruli produced by the action of adrenalin and may be regarded as a typical illustration of the changes described in this section.

These experiments clearly show that under the conditions described the number of glomeruli which receive blood in the frog's kidney is highly variable. When as a result of pithing, hemorrhage and operative trauma, the number of glomeruli receiving blood is small, a very large increase in this number can be accomplished by restoring blood volume either with

blood or with salt solution. When the experiment is conducted with minimal or no blood loss a considerable increase in the number of glomeruli which receive blood can be brought about by injection of urea, caffeine, glucose, sodium sulphate, small doses of pituitrin, or by section of sympathetic fibers. On the other hand, a decrease in the number of glomeruli which receive blood can be effected by adrenalin, large doses of pituitrin, or by stimulation of sympathetic vaso-constrictor fibers.

VARIATIONS IN THE CAPILLARY PATHWAY IN A GLOMERULUS. It has been possible to show also that the number of capillary loops through which blood cells are flowing within a single glomerulus is subject to variation in an analogous manner. Allusion has been made to the uncertainty which we felt early in this work as to the identity of the various circular arrangements of capillaries which we believed to be glomerular tufts. This uncertainty arose from the differing aspects of the capillary loops in different glomeruli, and is illustrated in the following sketches.

Figure 3 shows sketches of the capillary pathways in three glomeruli. In *a* the blood current was restricted to one widely dilated capillary through which slowly moved a column of densely packed cells: in *b* and *c* the pathway was similarly restricted but the capillaries seemed narrower; the corpuscles passed through singly and only at intervals. Flow when it occurred was rapid. Other capillaries in these tufts were indistinctly visible and contained here and there a motionless red corpuscle.

In figure 4 are shown three sketches of glomerular tufts in which blood flow was rapid, and in which the patent capillary pathway was much more complex. It is common to see in a single tuft one or more capillary loops in which the red cells move more slowly and in wide, dense column, while on all sides of it there are capillaries through which corpuscles pass more rapidly in single file (fig. 5, *c*). In the course of an hour's observation one may see that these relations change; the wider column of cells may become narrower so that the rapidity and character of flow throughout the tuft becomes more uniform. In glomeruli in which the branching of the afferent vessel is distinguishable, it is frequently found that the greater portion of entering blood cells passes in steady flow into one of three available channels, only a scanty flow entering the other two. Later observation of the same glomerulus may show that another one of the three has become the chief channel of glomerular flow. Steady constant flow in one loop, pulsating intermittent flow in another may be encountered in the same glomerulus. One can occasionally see abrupt changes in glomerular capillary flow, of which the following is an instance:[5] A glomerulus was encountered in which blood cells were flowing in one dilated

[5] This observation was made by Wearn and Richards during the course of the experiments described on pages 209 to 227.

capillary only, the rest of capsular space appearing empty. While watching, a second stream of cells made its appearance, and a few moments later a third, the second and third branching from the first close to its origin from the afferent vessel. Both of these were much narrower than the first, only one corpuscle passing at a time. Movement in the first was steady and uniform; in the second and third it was intermittent (not pulsatile), and this intermittence was roughly an alternating one—when one capillary was open the other was closed and invisible.

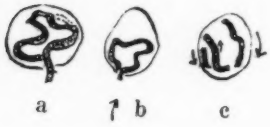


Fig. 3

Fig. 4

a (× 58—$\frac{1}{5}$ sec.) b (× 63—3 secs.) c (× 66—1 sec.)

Fig. 5. Photomicrographs illustrating different types of blood flow through different glomeruli. In *a*, blood flow was very rapid through both glomeruli, but in 1 the blood cells were closer together and in wider column than in 2. In *b* glomerulus 1 showed sluggish blood flow through apparently dilated capillaries, whereas in 2 a few corpuscles only shot through at infrequent intervals. In *a* is shown a tuft in which two capillaries appear dilated, while the rest of the tuft appears constricted. *a* and *b* well illustrate the normal variations in size of different glomeruli.

Not only is it possible, as described, to detect striking differences among different glomeruli in respect of the character of their capillary circulation; it is also possible to effect comparable changes in a single glomerular tuft. The following series of drawings (fig. 6), made with the aid of a camera lucida, represents the capillary pathways in a single glomerulus at different times as they were revealed by the corpuscles passing through.

Enough experiments of this type have been made to prove that those influences which are capable of increasing the number of active glomeruli

of the kidney are also capable of increasing the extent of the capillary pathway in a single glomerular tuft when this has been restricted: and that restriction of the capillary pathway in a tuft can be accomplished by those agencies which lessen the number of glomeruli through which blood is flowing.

Later in this paper experiments will be described which show that the character of the stream of blood cells which flows through a glomerular capillary may give a false impression of the state of constriction of the capillary. The passage of cells, one by one, into and through a capillary may lead to the belief that the capillary is constricted, when in fact only its entrance is narrowed—a fact, emphasized by Krogh, which we were slow to realize. This qualification, however, does not essentially alter the major conclusions to be drawn from the observations recorded.

INTERMITTENCE OF GLOMERULAR CIRCULATION. In many preparations we have observed that the flow of blood through the glomerular capillaries

Fig. 6. R. pipiens. Variations in capillary pathway within a single glomerulus. *a*, 5 minutes before an intravenous injection of 0.1 cc. of 10 per cent glucose: blood flow very slow. *b*, 10 minutes after glucose: blood flow still slow. *c* and *d*, 25 and 30 minutes after glucose; blood flow more rapid and cells less closely packed. *e*, 45 minutes after glucose: blood flow slow, cells densely packed. *f*, 9 minutes after intravenous injection of 0.5 cc. 0.7 per cent NaCl: flow more rapid and cells less dense. *g*, immediately after injection of 0.1 cc. adrenalin 1/100,000: blood flow very slow. *h*, 5 minutes after a *g*, blood flow rapid.

may show occasional interruptions. They are not synchronous with diastole of the heart; they are not regular in occurrence, and they do not involve all the glomeruli under observation in a single field to the same degree. In some instances blood flow stops and the corpuscles disappear from the capillaries; in others, on cessation of blood flow, the corpuscles remain motionless in the capillaries throughout the interruption.

In one preparation, intermittence in one glomerulus was timed for short periods as follows: on,[6] 15 seconds; off, 12 seconds; on, 27 seconds; off, 11 seconds.

In another: off, 13 seconds; on, 11 seconds; off, 14 seconds; on, 12 seconds; off, 12 seconds; on, 17 seconds.

The following arrangement was made to secure a graphic representation of this phenomenon:

[6] "On" and "off" mean blood flowing and not flowing respectively.

Five telegraph keys were set up along side the microscope, each connected with a signal magnet arranged to write on a smoked drum. Five glomeruli were chosen for observation in a single field, and to each glomerulus was assigned a key for signalling changes in its circulation. One of us, at the microscope, pressed the appropriate key whenever blood flow stopped or became appreciably slower in any glomerulus: the other recorded on the drum the meaning of the signals as they were given. From the five records thus made a chart was constructed which gives an approximate representation of variations in blood flow in five closely adjacent glomeruli (fig. 7). The broad black lines represent rapid flow and the narrower lines slower flow, often with restriction of channels through which flow occurred. Interruptions of the line represent complete interruption of flow.

It will be observed that while there are times during which blood stops flowing in all at once, there are others at which the flow in one is completely independent of that in the others.

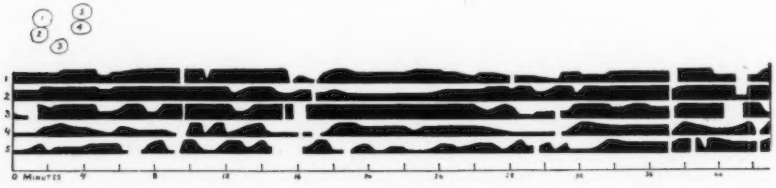


Fig. 7. Chart representing spontaneous variations in the flow of blood through five closely adjacent glomeruli, the relative positions of which are indicated by the sketch at the upper left hand corner. A broad line represents rapid flow, a narrow line slow flow and interruption of the line represents discontinuance of flow.

The following experiment shows that this intermittence of glomerular flow may be encountered after complete destruction of the spinal cord as well as of the brain.

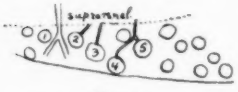


Fig. 8

January 8, 1921. R. pipiens, 50 grams, brain pithed.

Figure 8 is a sketch showing five glomeruli chosen for observation. The following notes were made:

2:50. Flow in kidney as a whole was very active. Glomeruli 2, 3, 4, and 5 showed almost continuous flow. No. 1 showed infrequent flow which was consecutive for a few seconds only.

2:54. No. 2 stopped, 5 slow, 3 and 4 rapid

2:58. Spinal cord pithed

3:00. No flow in any of the five

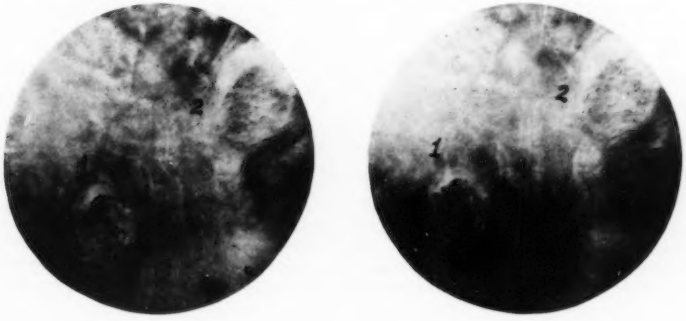
3:05. Flow begins actively in no. 4; an occasional red cell passes through no. 5; nos. 1, 2 and 3 show no flow.

3:18. 1 cc. of 0.65 per cent NaCl injected into ventricle. Blood flow much accelerated. Nos. 1, 3, and 4 began actively, then all stopped at once; then all began in order –1, 2, 3, 4 and 5; then all stopped together; then 4 alone began, then stopped; then 3 alone began, then stopped.

3:25—All began at once, then stopped at once; then 4 began alone with sluggish flow in 5 and 2; all stopped; 4, 2, and 3 began flowing in that order; 4 and 3 stopped; 2 continued, then all stopped.

In figure 9 two photomicrographs are shown which demonstrate spontaneous intermittence. At the time of making photograph *a* no blood was flowing through glomerulus 1, the cells shown in the upper part being motionless; 65 seconds later *b* was made. At this time blood was flowing rapidly throughout the entire tuft. The pictures indicate that the relations exhibited by 1 were reversed in the case of glomerulus 2.

In three series of experiments it has been possible to produce experimentally an intermittence of glomerular blood flow similar to that described in preparations in which it had previously been absent or incon-



a b

Fig. 9. R. catesbiana. Spontaneous intermittence. Picture *b* made 65 seconds after *a*. During exposure for *a* no blood was flowing through glomerulus 1: during exposure for *b*, active circulation occurred throughout the capillaries of 1. Magnification = × 63. Exposures, 5 seconds.

spicuous. This was accomplished by *a*, electrical stimulation of the central stump of the sciatic nerve in a frog which had been lightly curarized; *b*, electrical stimulation of the ramus communicans of the 5th or 6th spinal nerve; *c*, slow intravenous injection of adrenalin at a constant rate. Illustrative results are given below.

Afferent nerve stimulation. April 28, 1924. Large pipiens. 2.2 mgm. curara. Brain pithed: left sciatic nerve exposed in the pelvis, tied and electrodes applied central to ligature. Right kidney prepared for examination by transmitted light. Three glomeruli selected for examination, the afferent arterioles of two of which originated from one parent vessel. Stimulus was supplied by a Harvard induction coil with one dry cell, the secondary coil being set at 12 cm. from the primary and at an angle of 70°. The following chart (fig. 10) shows changes in blood flow.

Stimulation of sympathetic fibers to the kidney. April 29, 1924. R. pipens. Brain pithed; right kidney exposed for observation by transmitted light: 5th spinal nerve

on right side exposed and cut distal and central to origin of ramus communicans. The ramus, carefully freed from fascia, was laid across small platinum electrodes, supported by an adjustable holder fastened to the frog board. Except during periods of actual stimulation the ramus was protected from drying by cotton moistened with Ringer's solution. The electrodes were connected with a Harvard inductorium,

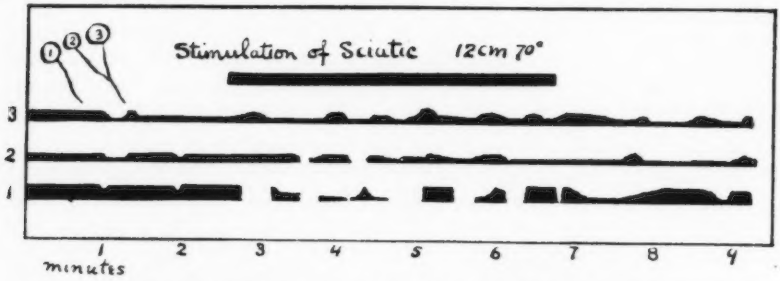


Fig. 10. Chart showing variations in blood flow in 3 glomeruli (relations shown in upper left hand corner) caused by stimulation of central stump of sciatic nerve in a curarized frog.

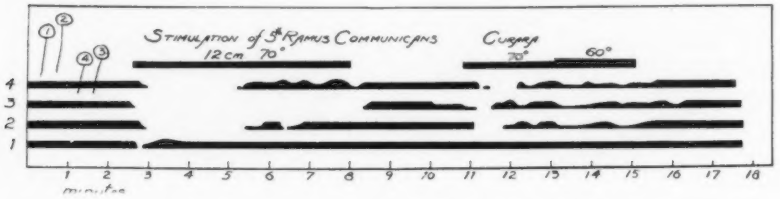


Fig. 11. Chart showing variations in blood flow through four glomeruli caused by stimulating the 5th ramus communicans.

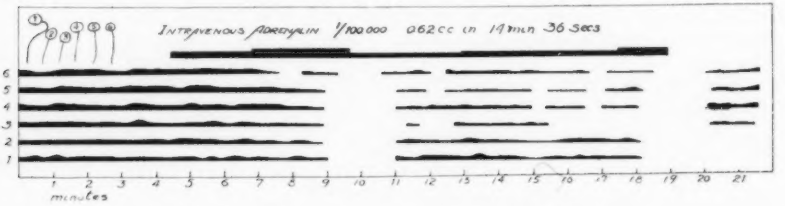


Fig. 12. Chart showing variations in blood flow in six glomeruli caused by the slow intravenous injection of adrenalin 1/100,000. Variations in the breadth of the injection signal line approximately indicate intentional variations in rate of injection.

(one dry cell), secondary coil being set at 12 cm. and at angles to primary shown in the charts. Twelve cm. and 60° gives a tetanizing current which is perceptible but not unpleasant when the electrodes are placed on the tongue.

Four closely adjacent glomeruli were chosen for inspection. Their afferent arterioles were traced for several millimeters in the direction of the aorta and were not found to join in that distance. The results are shown in figure 11.

Injection of adrenalin. April 30, 1924. R. pipiens, ♀. Brain pithed; cannula in anterior abdominal vein. Right kidney prepared for observation by transmitted light. Six glomeruli were chosen, the afferent arterioles of which could not be seen to join. 1:100,000 adrenalin chloride solution, made up in Ringer's solution without bicarbonate, was injected into the anterior abdominal vein, using a 1 cc. syringe, graduated in 1/100's, attached to the cannula by about 12 inches of small rubber tubing. The injection was made by the person who was watching the glomeruli. A device made by Dr. A. E. Livingston of this laboratory was used for maintaining a uniform injection at a sufficiently slow rate. The barrel of the syringe was firmly held in a metal support in such a way that the piston of the syringe could be pushed by turning the milled head of a screw which passed through a projection on the syringe holder. One hundred full turns of the screw moved the piston sufficiently to eject 0.70 cc. A metronome beating half-seconds stood near by and at every beat the screw head was rotated through a fraction of a turn. After a little practice the operation became quite automatic and gave a sufficiently uniform injection.

The results are shown in figure 12.

CONTRACTILITY OF THE GLOMERULAR CAPILLARIES. The fact that in a single preparation one may see some glomerular capillaries through which the red cells are moving in a wide dense column, while in others the cells pass rapidly through in single file; the fact that the one type of flow may be converted into the other by appropriate means; and the further fact that when blood flow through the glomerular tuft is arrested, either spontaneously or as a result of nerve stimulation, adrenalin injection or occlusion of the aorta, its capillaries often become optically empty, appeared to furnish prima facie evidence of contractility of the glomerular capillaries analogous to that which has been demonstrated in other capillary areas. The question is complicated, however, by a number of factors.

Owing to the difficulty of seeing the actual capillary wall most of our impressions of glomerular capillary calibre have been gained from the appearance of the columns of cells passing through rather than from estimate or measurement of their true diameter. It is obvious that such inferences may be erroneous. When relatively few corpuscles at wide intervals are seen moving rapidly through a capillary the first impression created is that the capillary itself is narrow. Yet, in such a capillary, if the movement of the stream becomes slow enough to permit close observation of a single corpuscle it may be seen to rotate and to bump from side to side as it could not do if it were being squeezed through a narrow tube. One can frequently see that corpuscles, sausage-shaped in their passage through the afferent arteriole because of its constriction, reassume their characteristic oval form when they enter the glomerular capillary. Hence in a glomerular tuft, through one of whose capillaries flows a wide stream of closely packed cells, while through another the cells pass more infrequently and in single file, we may conclude that the opening from the afferent vessel into the former capillary is wider than that into the latter, rather than that the diameters of the two capillaries throughout their

course are greatly different. When a glomerulus is encountered, through all the capillaries of which cells move in single file and are relatively widely spaced and when the blood stream in the afferent arteriole shows similar wide spacing of corpuscles, the assumption may be made that the character of the glomerular flow is being determined by constriction of the afferent arteriole or of its parent artery and not necessarily by constriction of the glomerular capillaries.

Changes in glomerular capillary caliber may be induced by changes in extra- and intra-capillary pressure. Extra-capillary pressure would appear to be a more important influence in relation to capillaries of the glomerular tuft than in capillaries elsewhere because of the existence of the highly elastic Bowman's capsule in which they are inclosed. When mechanical pressure is exerted upon the exterior of the capsule the tuft can be seen to shrink and blood flow through it may cease: when a capsule, distended by the fluid in it, is ruptured the tuft expands. In the one case we assume that the caliber of the glomerular capillaries is lessened, and in the other increased.

Changes in glomerular capillary caliber can also be induced by the changes in intra-capillary blood pressure which may result from changes in arterial pressure, in the caliber of afferent or efferent vessel, or in the state of tonus of the points of origin or reassembly of the capillaries of the tuft.

The above considerations indicate the difficulties with which one is confronted when attempting to study either intrinsic glomerular capillary contractility or the influence of nervous stimuli. They are not different in kind from those encountered in the study of capillaries elsewhere, but in some respects are greater in degree because of the greater difficulty in clearly seeing the capillaries, because of their different mode of origin from the arteries, and because of the capsule which envelops them.

Two observations only can be cited by us in support of the view that the glomerular capillaries are intrinsically contractile, and both are explainable on other grounds. One has already been mentioned on page 186. A glomerular tuft was seen in which only one capillary was distinguishable. Through this a wide stream of rather densely packed cells flowed during the whole period of observation. Presently a second capillary opened, and conveyed a narrow stream of more widely spaced cells through the tuft. Later a third appeared, similar to the second, and for several minutes these two alternated in flow. When flow ceased in either, it became invisible because no cells remained stagnant in it. In this instance conditions referable to extra-capillary pressure and to pressures in afferent and efferent vessels were the same for the three capillaries, and it seems certain that the peculiarities of behavior of the second and third in relation to the first were the results of alternating contraction and re-

laxation. There is no evidence, however, that the contraction and relaxation involved the whole course or any considerable part of the course of either capillary. Contraction and relaxation of a ring at the origin of each capillary might have been responsible for what we saw.

The second experience indicative of contractility of glomerular capillaries was encountered in an experiment in which the abdominal aorta above the origin of the renal arteries was clamped. A glomerulus was watched in which, before clamping, a wide, densely filled capillary was patent. After clamping the aorta, the blood current in this capillary came to rest and then the capillary slowly emptied itself in a fashion quite different from that usually seen. It appeared that contraction began at the distal end and progressed slowly toward the proximal end; the corpuscles could be seen escaping through the narrowed portion. In this instance the entire capillary eventually became contracted. Figure 13 shows three sketches made directly after this observation to illustrate the progress of emptying.

Fig. 13

A large number of attempts have been made to detect narrowing of glomerular capillaries during stimulation of the sympathetic fibers to the vessels from which they branched and during the action of adrenalin, intravenously injected. While it has never been possible to see clearly the glomerular capillary wall throughout any large fraction of its course, it has frequently been possible to obtain a sharp focus on very short portions, particularly at points where the capillary bends. In no case, thus far, has indisputable evidence been encountered that contraction occurred.

FURTHER OBSERVATIONS ON BLOOD FLOW THROUGH GLOMERULAR CAPILLARIES. Previously in this paper we have repeatedly directed attention to the fact that the moving columns of blood cells in the various glomerular capillaries in a single preparation may present widely different appearances: in one they move slowly and are packed together, in another the stream is rapid and narrow, in a third they are in single file with considerable space between each two cells, while in a fourth a single cell, or groups of several cells, may pass through rapidly or slowly at intervals as long as several seconds. It will be recalled that Cohnstein and Zuntz (4) made observations which showed that the red cell count of blood collected from large vessels could be markedly increased by stimulation of the cord (vaso-constriction) and decreased by vaso-dilatation. In analyzing this phenomenon they subjected the web and the omentum of the frog to direct observation and saw some single capillaries through which a red corpuscle "shot" now and then; and by constrictor nerve stimulation they were able to produce this type of flow through arteries which before stimulation were as wide as 30 to 50 μ. They postulated the existence of capillaries (*vasa serosa*) through which only plasma flowed, their caliber being too

small to permit the passage of cells. Our observations on the proportion of cells to plasma in different glomerular capillaries are precisely analogous to theirs. Inspection alone is sufficient to show that the proportion of cells to plasma in the blood flowing through different glomerular capillaries is not uniform, and we may at once conclude that urine is simultaneously formed in different glomeruli in the kidney from blood of different compositions in so far as plasma and cells are concerned. Whether it will ever be possible to evaluate such a factor as this in estimating the function of a kidney can hardly now be said.

Our observations have an interesting bearing on the question of the existence of *vasa serosa*. Krogh observed that when a small artery branching from a larger vessel was made to contract, a stage of its contraction could be identified at which plasma alone was able to pass through (5). This "plasma-skimming" he attributed to the existence of a marginal zone of plasma in the blood stream as it flows in the larger vessel. In a later discussion under the caption "The non-existence of "vasa serosa", he expressed the belief that only under extraordinary circumstances and for short periods can capillaries admit plasma without corpuscles (6). "When a capillary containing one or more red corpuscles contracts to such a degree that the corpuscles cannot pass along, they will be squeezed into a form which fills up entirely the lumen of the vessel and prevents any passage of plasma along it." In the previous section we have presented the view that the orifice of origin of a glomerular capillary may be so constricted as to bar out cells but not plasma. The following observations give proof of the reality of this possibility:

i. Two glomeruli in one kidney were chosen for simultaneous observation: one showed steady flow of rather densely packed corpuscles through all of its capillaries; in the other the cells passed more infrequently, in single file and were more widely spaced; 0.4 cc. of a solution of Janus green B, 1:2000, was injected into the aorta above the renal arteries. The resultant coloration of the two glomerular tufts appeared to be equally prompt and equally intense. Had the volume of flow of fluid through the two tufts differed as greatly as the difference in cell content of the tufts indicated, marked difference in time and intensity of staining might have been expected.

ii. In another frog, one glomerulus was observed through which cells passed infrequently. An intra-aortic injection of Janus green B was begun. Almost immediately, just when the tuft began to appear green, flow of corpuscles through it ceased, but the intensity of coloration of the tuft continued to increase.

iii. In a large catesbiana, a glomerulus was seen in which the capillaries appeared to be empty. Now and then one or two corpuscles entered, floated lazily through and disappeared. A suspension of washed sheep's corpuscles in salt solution was injected into the aorta through a cannula inserted into the stump of the coeliac axis. (Sheep's red cells are round and approximately 5 μ in diameter; frog's cells are oval, approximately 23 $\times$ 16 μ.) Immediately there appeared throughout the entire glomerular tuft a cloud of sheep's corpuscles in which no frog's corpuscles could be distinguished. Clearly the pathway to this tuft, too narrow to admit the large corpuscles of frog's

blood, was wide enough to admit the small, sheep's cells: hence plasma may have been flowing through it when it appeared empty.

These observations, together with those in the preceding section, indicate that glomerular capillaries may at times approximate to the "vasa serosa" of Cohnstein and Zuntz, and they show that this may come about through the existence of a constriction limited to the origin of the capillary.

Tarchanoff (7) in his paper on the contractile elements of blood and lymph capillaries made the statement which we regard as highly significant, that on applying an electrical stimulus to the capillaries of a frog's nictitating membrane constriction appeared much sooner at the angle of origin of capillary from artery than in other "spindle elements" of the capillary. We have a number of observations (Schmidt) on arterioles and arterioles and capillaries in the gastrocnemius muscle of the frog made before and during the action of adrenalin (1:100,000), intravenously injected in dosage of the order of 0.1 to 0.3 cc., which repeatedly indicated that the constriction caused by this substance is often more conspicuously exerted at the branching of the vessels than along their course. These experiments have not been extended sufficiently to permit us to maintain that this last statement is applicable to constrictor control over the smaller vessels in general. If further work should show that this is true, we would have a better understanding of the fact that obstruction by massed corpuscles such as Krogh's postulate implies is so rarely encountered in direct observation of normal vessels and a means would be revealed whereby, in the small blood vessels, viscosity of blood changes with alterations in the caliber of the vessels.

The observations cited in this section may be thought to invalidate those in sections 1 and 2. The possibility of the existence of streams of plasma without corpuscles through glomerular tufts and their consequent invisibility by the methods we have used certainly does increase the probable error of our counts of "active" and "inactive" glomeruli, though probably to no great degree. Since constriction of such a grade as to admit plasma and not cells must be regarded simply as one phase between complete closure and wide dilatation, it would be absurd to assume that all invisible glomerular capillaries were traversed by streams of plasma. The observations of Khanolkar bear out this statement.

ACTION OF SMALL DOSES OF ADRENALIN. The question which originally led to all of the experiments described in this paper concerned the action of minute amounts of adrenalin injected into the blood stream. We hoped to be able to see whether or not the smallest effective doses caused narrowing of the efferent vessel with distention of the glomerular tuft. Accurate determination of the diameter of the efferent vessel proved to be difficult, so we turned to measurements of the diameter of the capillary tuft.

Adrenalin in dilutions varying from 1:200,000 to 1:2,000,000 was injected into the anterior abdominal vein in amounts ranging from 0.05 cc. to 0.3 cc.

Consistent results were obtained in some twenty instances following the intravenous injections of an amount of adrenalin of the order of 0.1 cc. of a 1:1,000,000 dilution. The following expressions occurring in our records of various experiments are typical:

"Tuft became more full of cells, flow through its capillaries was slower as though blood was being presented to it as rapidly as before, but was leaving more slowly."

"Unmistakable increase in diameter."

"Fairly distinct swelling, tuft engorged with cells, flow in tuft slower after injection."

The record of one experiment is as follows:

R. pipiens, ♀, 35 grams. Brain pithed; ovaries excised; right kidney arranged for observation by transmitted light; cannula in anterior abdominal vein for injections; monocular microscope, Leitz no. 2 objective, micrometer eyepiece no. 2., Magnification 36 diameters. Diameter of glomerular tuft selected for observation = about 218 μ. Injected 0.1 cc. 1:1,000,000 adrenalin. First effect, occurring about 15 seconds after injection, is slowing of glomerular blood flow, greater packing of cells in capillaries, and swelling of tuft (about 218 μ to about 229 μ). Two repetitions gave swelling from about 229 to 249 and about 229 to 260 μ respectively. Controls in which 0.1 cc. of 0.65 per cent NaCl was injected showed no appreciable change. In each instance this apparent engorgment of the tuft was followed (25 to 35 seconds after injection) by lessened inflow of blood via the afferent vessel and the diameter of the tuft decreased.

The conclusion to which we came as a result of these measurements was that the earliest effect which is exerted by minimal effective dosage of adrenalin on the size of the glomerular tuft is such as might result from constriction of the efferent arteriole. It is obvious, however, that a similar effect could be produced by accelerated inflow of blood into the glomerulus and in a number of experiments such an acceleration of inflow was observed. This was attributed to cardiac stimulation and also to the possibility of a greater constriction of other vascular areas than those under direct observation. It seems certain that an experiment on the frog's kidney can be made in which blood flow shall be kept constant through the kidney during the action of adrenalin and in which therefore such sources of confusion will be eliminated. Until such an experiment can be made decision must be postponed, and all that can now be said is that the phenomena as seen by us in the frog's kidney are not in disaccord with the view that adrenalin in smallest active amounts causes a constriction of the efferent vessel.

The effect of somewhat larger doses of adrenalin than those mentioned above is of interest in connection with our earlier observations on the glomerular circulation. It has already been stated that constrictor doses

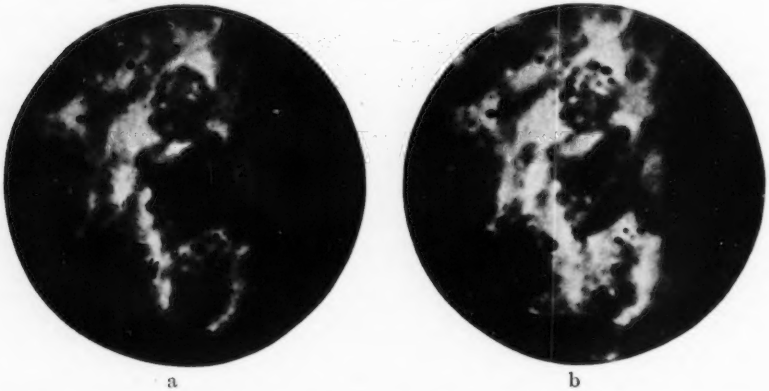
of adrenalin may diminish the number of glomeruli which receive blood and may lessen the number of patent capillary loops in a single glomerulus which receive blood. It has just been stated that immediately after the initial engorgement and swelling of the tuft which follows 0.1 cc. of 1:1,000,000 restriction of inflow of blood and decreased size of tuft may be observed. It can then be seen that the corpuscles moving in the arteriole which supplies the glomerulus under observation are further apart, and their rate of progression is slower. If one of the glomerular capillaries appears dilated, another narrow, it is found that flow of the narrow stream stops while that of the wide stream continues.

If the dose of adrenalin is slightly increased, e.g., 0.1 cc. of 1:500,000 or 1:200,000, flow in the glomerulus as well as in its arteriole stops and the vessel becomes empty. It is striking, however, that while this stoppage of flow may occur in one glomerulus and its arteriole of supply, another glomerulus in close proximity to it may keep on flowing on with apparently unaltered rapidity. It is clear that the degree of susceptibility of different vessels in the kidney to adrenalin is not the same. These changes are illustrated in figure 14.

The interruption of the circulation in a glomerulus which follows this somewhat larger dosage of adrenalin is not due to obstruction in or at the glomerulus itself. The slowing and cessation of blood flow is first to be observed in the arteriole leading to it.

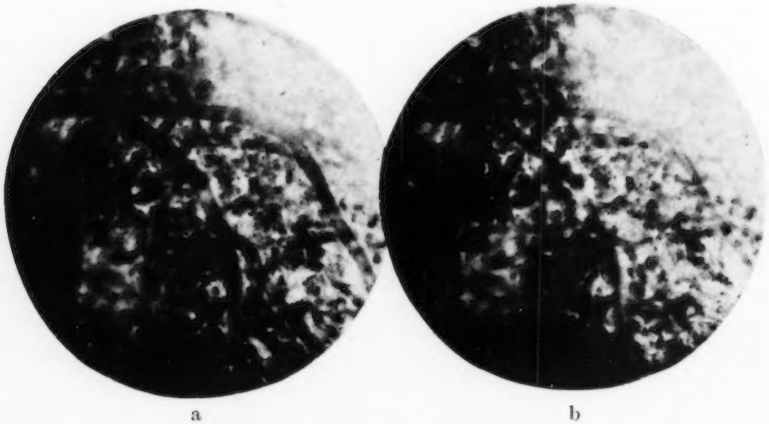
Pictures have been made of small arteries and arterioles in the kidney before and during their response to intravenously injected adrenalin. In making figure 15 arteries 0.05 to 0.1 mm. in diameter were observed: the constrictor response is well shown. The reaction of smaller vessels is shown in figure 16 in which arterioles less than 0.03 mm. in diameter were chosen. A field of kidney surface in which the smallest arterioles and afferent arterioles were conspicuous was photographed before, during, and after the intravenous injection of adrenalin without change of focus. On the prints from these negatives, the shadows of the arterioles were carefully blackened with india ink and the rest of the picture covered with Chinese white. The reproduction of a photograph of these shown in the figure gives a good representation of the fashion in which these small vessels contract. At first all were narrowed and some disappeared: then, the injection still continuing, dilatation followed by constriction occurred. Shortly after the injection ceased all of the original vessels reappeared, some distinctly dilated, and in addition minute arteries not previously seen were found in the field. While not too much reliance is to be placed upon the complete accuracy of this method of recording fleeting changes, yet the impression which this record creates quite faithfully reproduces that gained by direct vision. Our experience indicates that changes completely similar to these can be induced by electrical stimulation of sympathetic fibers to the kidney.

DISCUSSION. Further discussion of the problem of change in size of the glomerular tuft brought about by the intravenous injection of very minute amounts of adrenalin is unnecessary at this time. It has been pointed out that our results are in harmony with the view that low con-



a b

Fig. 14. R. pipiens. Action of adrenalin on a group of four glomeruli. Magnification = × 56. Exposures, ½ second. Photograph *a* was made 1½ minutes before the intravenous injection of 0.15 cc. 1:100,000 adrenalin. Circulation was active in all glomeruli. *b* was made 1 minute after the injection. No blood was flowing in glomerulus 3; the circulation was diminished in the others.



a b

Fig. 15. R. catesbiana. Action of adrenalin on arterioles 0.05 to 0.1 mm. in diameter. Magnification = × 30. Exposures, 2 seconds. Photograph *a* was made 20 seconds before beginning an intravenous injection of 1:100,000 adrenalin at the rate of 0.2 cc. per minute. *b* was made 70 seconds after the beginning of the injection.

centrations of renal vaso-constrictor substances may produce a more effective constriction of the efferent pathway than of the afferent. The limitations of our observations have been mentioned and the plan of an experiment which may be crucial has been outlined. It seems obvious that we require more information concerning the details of innervation of the glomerular capillaries, not only throughout their course, but especially at their points of origin and reassembly.

The behavior of the glomerular circulation as revealed in the other experiments described is deserving of further comment. The discovery

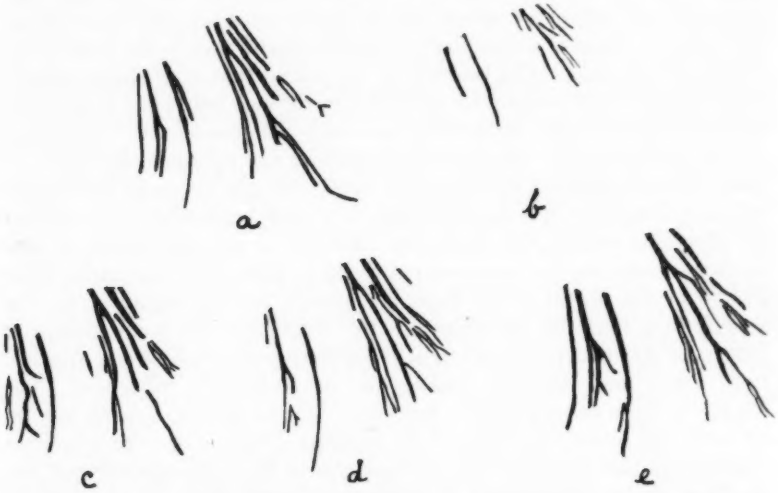


Fig. 16. R. catesbiana. Action of adrenalin on arterioles less than 0.03 mm. in diameter, including afferent vessels. *a* was made 26 seconds before beginning an intravenous injection of 1:50,000 adrenalin at the rate of about 0.1 cc. per minute. *b*, *c* and *d* were made during the injection at intervals of 34 seconds, 84 seconds and 2 minutes 24 seconds respectively, after its beginning; *e* was made 2 minutes after stopping the injection, the total amount injected being 0.3 cc.

that the method of Malpighi, as developed and brilliantly applied by Krogh in recent years, may be adapted to the kidney of the living frog in such a way as to render the glomerular circulation easily accessible to direct observation, is important in that it provides not only a fresh field of investigation, but also a readily available means of student instruction.

Our observations show that marked alterations occur spontaneously or may be induced experimentally in the glomerular circulation. They appear to result not so much from changes in the glomerular capillaries themselves as from changes outside of these. Evidence that glomerular

capillary contractility exists is very scanty. Actually the only experiment in which it appeared to be established is the one in which occlusion of the aorta was followed by the progressive emptying of a dilated glomerular capillary from its distal end backward. Here it could be seen clearly that the capillary collapsed; but it is by no means certain that this was not the result of negative pressure during cardiac diastole exerted from the venous side. The opinion to which we have been driven during the later stages of this work is quite different from that held earlier (9), before we came to recognize the possibility of illusions concerning capillary caliber which might result from observations of the processions of cells through the capillaries and before we recognized the important part which the size of the orifice of origin of a capillary or arteriole might play in the proportion of cells and plasma passing through it. It is clear that everything we have seen relating to differing modes of blood flow through individual glomerular capillaries is explainable without the necessity of assuming active participation of the capillaries: this statement includes the different characters of blood flow encountered under control conditions, the apparent opening or widening of capillaries under the influence of renal vaso-dilators, the apparent closure or constriction under the influence of nervous stimuli or constrictor substances, the apparent emptying of glomerular capillaries after closure of the aorta: all of these phenomena can be explained by the assumptions that the arterioles are the structures which are primarily influenced and that points of origin of capillary from arteriole and arteriole from artery are especially susceptible to constrictor influences. The former of these assumptions is supported by our failure in many careful efforts to see actual narrowing of the capillary diameter or to gain indirect evidence of this in distortion of corpuscles in their passage through the capillary during stimulation of sympathetic nerve fibers to the kidney, during the action of adrenalin and during the moments preceding complete arrest of the circulation following aortic obstruction; recognition of change in diameter of arterioles is easy under the same circumstances. The latter assumption is supported by the fashion in which interruption of flow of red cells from a parent artery into an arteriole occurs during adrenalin constriction and by the complete absence of any appearance of obstruction of arteries or arterioles by corpuscles during the action of a constrictor agency. On the contrary, during the development of such action, one can see the cells becoming more and more widely spaced, until each two corpuscles may be separated by an intervening column of plasma many times the length of a corpuscle, an apparent dilution of corpuscles occurring which calculation shows would be impossible were the relations of corpuscles to plasma the same as in blood drawn from a great vessel. The only approach to such obstruction which we have seen is the congested engorgement of glomerular capillaries which was observed immediately

after the injection of very minute amounts of adrenalin—an effect which we attribute to constriction of the efferent vessel: this may well be exerted at the point of reassembly of the glomerular capillaries to form the efferent vessel.

Recent work on the physiology of the capillaries in other regions than the kidney has supplied evidence of intrinsic contractility and of nervous control so convincing that we have regarded our failure to find similar evidence in this work with some misgiving. It is of interest to note that Dale and Laidlaw found that histamine, in contrast to other organs tested, regularly caused decrease in volume of the kidney in the cat (10). Concerning the innervation of glomerular capillaries we have only the statement of von Smirnow (11) that non-medullated fibers enter the Malpighian body along with the afferent vessel and are related to the glomerular capillaries (zu den Gefässchlingen der Malpighi'schen Knäuel in Beziehung stehen).

Until other evidence than we can now supply is forthcoming we shall regard the circulation through the glomerular capillaries as largely if not wholly determined by influences which do not include their active contraction or relaxation.

Proceeding from this decision, we may consider the observed alterations in glomerular circulation, regarded largely as manifestations of change in arterial or arteriolar blood flow, in relation to the function of the kidney as a whole. Account must be taken of the following facts: the number of glomeruli through which blood visibly circulates at any moment is not necessarily the total number of glomeruli in the kidney: the number of capillaries in a single glomerulus through which blood visibly flows is not necessarily the total number of capillaries in the tuft. Both may be increased by influences which cause vaso-dilatation of the kidney or decrease viscosity of the blood: they may be decreased by vaso-constrictor agencies. Among the former are to be counted section of the sympathetic nerve supply to the kidney, injection of isotonic NaCl solution, injection of various salts, glucose, urea, caffeine and pituitrin (minute amount). Among the latter are to be included afferent nerve stimulation, direct stimulation of the sympathetic nerve supply to the kidney, hemorrhage, injection of adrenalin and of pituitrin (large amount). Account must also be taken of the fact that often the visible flow of blood through different glomeruli and through different capillaries of the same glomerulus is intermittent. We hope to show that these facts are closely inter-related and that they constitute evidence of the manner in which the function of the kidney is adjusted to the requirements of the organism which was not available before these experiments were first described.

Since the time of Bowman and Ludwig it has been recognized that alterations in the renal circulation play an important part in the adjust-

ment of renal function to excretory requirements. But throughout the literature the assumption is implicit that the whole kidney acts as a unit and that alterations in the renal circulation affect all of the vessels of the kidney alike.[7] The discovery that concomitantly with renal vasodilatation and vaso-constriction the number of glomeruluar capillaries through which blood flows undergoes increase and decrease shows that to the recognized major factors in glomerular urine formation another must be added, viz., extent of glomerular capillary surface. The recognition of this factor assists our understanding of a number of phenomena of renal physiology such as the magnitudes of response by the kidney to comparatively slight changes in composition of blood; frequent failure of many glomeruli to be influenced by dyes or by toxic substances intravenously injected, discrepancies between oncometric measurements of kidney volume diuresis, and, as Hermann clearly pointed out, the capacity of two kidneys to elaborate urines of different compositions from a common blood supply.

If this conception be true, that a fraction of the total glomerular apparatus of the kidney may be active at any one time and that the size of the fraction is adjustable to the excretory requirement of the body at that time, the question may well be asked, how it is that the glomerular structures are protected from the damaging influence of prolonged cessation of blood flow through them. It has long been known that a transient interruption of the renal blood flow is followed by albuminuria. Good evidence exists that the albuminuria is of glomerular origin (12): obviously the glomerular capillaries lose their impermeability to plasma

[7] One striking exception to this statement is to be found in the early literature of the physiology of the kidney. In 1859 Hermann, writing from Ludwig's laboratory concerning differences in volume and composition of urine eliminated by the two kidneys of the same animal, made the following statement:

"Versucht man diese Folgerungen mit der Filtrations- und Anziehungshypothese zu vergleichen, so dürfte sich etwa sagen lassen: Zu der Filtration passt es vollkommen, dass sich die Ausscheidung des Harns und des Harnstoffes gleichzeitig erhöhen und dass die Harnstoffprocente des Harns der Niere geringer sind, welcher die meiste Flüssigkeit liefert. Um aber auch das entgegengesetzte Vorkommen aus der Filtrations-Hypothese zu erklären, könnte man statt irgend welcher verwickelteren Annahme einfach unterstellen dass die Ungleichheiten der Harnabscheidung auf beiden Nieren nicht allein in einer verschieden starken Absonderungsgeschwindigkeit auf der Flacheneinheit begründet sei, sondern auch daher rühren könne, dass die Niere nicht zu allen Zeiten auf ihrer ganzen Flache Harn abscheide. Stellt man sich vor, dass die Niere einer Seite überall mit geringer Geschwindigkeit absondert, während in der andern ein Theil ruht, und ein anderer Theil rasch absondert, so wird der Harn in der ersteren länger verweilen und concentrirter werden als in der letzteren. Also kann trotz gleicher Beschaffenheit des Blutes in beiden Nieren doch der Harn auf der einen Seite weniger reichlich und zugleich harnstoffärmer fliessen als auf der andern." (Sitzungsber. d. k. Akad. d. Wiss. zu Wien. Math.-Naturwiss. Kl., 1859, xxxvi, 358.)

proteins as the result of cessation of blood flow. Yet if we are to assume that a fraction only of the glomeruli of the kidney is sufficient to take care of the glomerular elimination under ordinary circumstances, we must explain how it is that the remaining fraction escapes damage, and that urine eliminated at the beginning of a profuse diuresis does not contain albumin.

The explanation which we suggest is in large part derived from our observations of intermittence of glomerular blood flow. A group of glomeruli are watched. Flow in one or two ceases for a time, while it continues in the others closely adjacent: then some of the latter cease, while the former resume. The several interruptions are irregular in time so that the field inspected presents the appearance of lively irregular intermittence of the glomerular circulation. The phenomenon is not commonly to be observed in the pithed frog which has been rendered highly diuretic, and in which, following the injection of relatively large amounts of salt solution, the renal blood flow is very rapid. But glomerular intermittence can regularly be produced in such a kidney by gentle faradic stimulation of the sympathetic fibers supplying the kidney, by central stimulation of an afferent nerve in the curarized frog, or by the slow continuous injection of adrenalin into a vein.

Rhythmic spontaneous contractions of arteries and arterioles have long been known (13). In none of the descriptions save that of Langley have we found intimation that these spontaneous contractions are associated with an irregular intermittence of arteriolar blood flow similar to that which we have observed in the frog's kidney. In none has the kidney been the subject of study in this connection.

Langley states (14):

> As previous observers, I have found that spontaneous contractions (in the arteries) depend chiefly upon the central nervous system, but that they may occur after its destruction and after section of the sciatic nerve; that the contraction is often unequal in different arteries of the web, and even in different parts of one artery. . . . . When a nerve has but a few vaso-motor fibers, stimulation of it never, so far as I have seen, causes equal contraction in all the arteries of the web; commonly one or two contract well and others not at all. . . . . Even when a nerve has a good and widespread vaso-motor effect the arteries do not usually contract equally either in rate or in extent. It has been mentioned above that spontaneous contractions are commonly unequal in different arteries, and that a contraction tends to lower irritability for a time; probably then spontaneous contractions tend to cause inequality of contraction on nerve stimulation.

These sentences contain the intimation that the vessels supplied by a certain efferent nerve may show irregular differences in degree of reaction to stimulation of that nerve and they supplied the direct impetus for undertaking the experiment of stimulating the renal sympathetic fibres. The results have been stated and unambiguous evidence has been collected

that constant stimulus, either nervous or chemical, may result in the intermittent response. Closure of an arteriole results in oxygen-want in the arteriolar wall. Deprivation of O_2 is known to cause prompt and marked dilatation of arteries, arterioles and capillaries (15). In the frog's kidney the afferent arterioles are very small—of capillary size. It is difficult not to believe that an oxygen exchange can take place through their walls. If this is so, then the dilator effect of substances formed in metabolism under the influence of deficient oxygen will be added to the direct effect of oxygen deficiency upon the muscle of the arteriole (16). Hence, it is evident that at the instant when closure of a renal arteriole occurs as the result of nervous or chemical constrictor stumulus, influences begin to develop which are dilator in effect and which presently, assuming the constrictor agency to be not too great, cause relaxation of the vessel wall and resumption of blood flow despite the continuance of the stimulus. Renewed supply of oxygen restores the ability of the artery to respond to the constrictor stimulus and thus an intermittent response is secured. We have repeatedly seen that an arteriole, closed in response to stimulation of its nerve fibers, remained closed for a minute, then, the stimulation still continuing, it opened and now its caliber was markedly greater than before the stimulus. It seems obvious that a series of such events involving a group of arterioles which have a common constrictor nerve supply could not take place in unison. In the antagonism ther between the constrictor influence of sympathetic nerve stimulation or of constrictor substances of which adrenalin may be regarded as typical, and the dilator influence of oxygen deficiency directly or indirectly exerted we see the chief element in the explanation of intermittence of glomerular blood flow.

The main features of this explanation to which the experiments have led are by no means new, save in their application to the circulatory events in the kidney. Thus, Roy and Graham Brown (17) discussing the dilatation of arterioles and capillaries which results from anemia (first described by Cohnheim) suggested the probable great importance of such dilatation in regulating the peripheral circulation, assuming that this is produced because of "diminution in the amount of O or other constituent of the blood . . . . or an increase in CO_2 or other product of the chemical changes of living tissue elements." A more specific conception of the manner in which the peripheral circulation is regulated in a tissue in accordance with its needs is presented by Gaskell in his paper on the action of alkali and acid on heart and vessels (18). "Throughout the whole vascular system, then, there is evidence to show that its normal tonicity and rhythmical action are dependent in part upon the due oxygenation and alkalinity of the fluid supplied to its muscular tissues, while, on the other hand, the tonicity cannot be maintained or the rhythmical action continued when that fluid is overcharged with such products of

tissue metamorphosis as lactic and carbonic acids." Dale and Richards (19) in discussing the relation which their study of the capillary action of histamine might bear to conceptions of peripheral circulatory control suggested that metabolic products with histamine-like action arise in the tissues, and exert upon capillaries an action antagonistic to those constrictor influences (possibly adrenalin) which are responsible for normal capillary tonus.

Krogh (20) clearly expressed the view that closure of a capillary causes lessening of its tonus and hence, sooner or later, the capillary must relax, readmit blood and so regain tonus. Thus alternation is produced. The similarity of our reasoning concerning intermittence of the renal arterioles to his concerning that of capillaries is obvious. While Krogh denied the causal influence of lack of oxygen in the capillary dilatation following ischaemia in the frog's tongue and web, substituting lack of the pituitary hormone, we believe that the known relaxing effect of oxygen deprivation on arterial muscle under conditions in which hormones can play no part together with the fact that glomerular intermittence is so clearly an arterial and arteriolar phenomenon make it unnecessary to introduce participation of the pituitary hormone into our explanation. In this connection it may be stated that pituitrin in dosage of the order of that studied by Krogh produces dilatation of arterioles of the frog's kidney.

Accepting the conception that conditions may normally exist in which participation of all the glomerular units in the function of the kidney is not required and that in these conditions a certain number are inactive we must assume that an intermittence of glomerular flow is operative at such a rate as to prevent cessation of circulation through any one glomerulus for so long a time as to cause damage to its endothelium.

As has been mentioned, total occlusion of the renal artery for a short time is followed by albuminuria. Intermittence of the glomerular circulation, brought about in the fashion described, explains how a portion only of the glomerular apparatus may be inactive without damage to the structures concerned. It might be argued that the intervals of normal glomerular intermittence must be of shorter duration than the shortest time of occlusion of the renal artery which is sufficient to cause albuminuria. Such an argument is fallacious. When the renal artery is occluded, oxygen starvation of the entire kidney results. When a certain number of afferent arterioles are closed only localized areas suffering oxygen deficiency are produced. Diffusion of oxygen from neighboring areas in which circulation is active may occur to such an extent that the time during which an arteriole may remain closed without damage will be longer than at first thought seemed possible.

There is another possible element in the production of intermittence which is worth mention. For a long time it was the only explanation

which occurred to us. It concerns differences in extra-capillary, intra-capsular pressure. When one examines a number of glomerular tufts in a kidney, he sees that the degree of distention of the different capsules is very different. It is clear that the values of extra-capillary pressure within different capsules are widely different. In any tuft in which intra-capillary pressure is only a little higher than extra-capillary pressure, blood flow will cease if general arterial pressure should fall so that intra-capillary pressure is lower than extra-capillary. In a neighboring capsule in which extra-capillary pressure is low such a lessening of arterial pressure would have little effect on glomerular flow. One reason for thinking that this explanation is not important in explaining glomerular intermittence as we have observed it is to be found in the fact that in normally occurring intermittence the flow in arterioles is intermittent: whereas when the capsule is compressed so that flow ceases because of high extra-capillary pressure the arteriole remains filled with blood.

SUMMARY

1. The glomerular circulation of the frog's kidney can easily be made accessible to direct microscopic observation.

2. The number of glomeruli through which blood flows and hence which function at any one time may be a fraction only of the total number of glomeruli in the kidney. This fraction is susceptible of increase by various vaso-dilator agencies and of decrease by various vaso-constrictor agencies.

3. The number of capillary pathways within a single glomerulus is variable in analogous manner.

4. Intermittence of glomerular circulation can frequently be observed. It can be produced by reflex constriction of the renal vessels, by electrical stimulation of sympathetic fibers to the kidney and by slow intravenous injection of adrenalin. It is thought to be due to the antagonism between nervous or chemical constrictor influences and the dilator influence of oxygen deficiency which begins to be manifest as soon as circulation ceases in the small arterioles. It is regarded an essential factor in the capacity of the kidney to utilize a fraction of its glomerular equipment without damage to the rest.

5. No conclusive evidence has been collected to show that the described variations in the glomerular circulation are due primarily to contraction or relaxation of the glomerular capillary wall. They appear to be the result of changes in the arterioles.

6. Phenomena have been encountered which lead to the conclusion that structures situated at the origin of the glomerular capillary from the afferent arteriole may not only exhibit spontaneous contractility, but may be more susceptible to constrictor influences than is the rest of the

muscle of the wall of the arteriole. Observations of arterioles in striated muscle and in the kidney have been made which suggest that in general the small arterioles constrict more readily at their points of branching from parent vessels than at other points. Such a view is in harmony with observations of Cohnstein and Zuntz, of Krogh and with our own finding concerning apparent dilutions of blood of varying degrees which can be seen in small arterioles and in glomerular capillaries.

7. Adrenalin, introduced into the circulation in amounts suited to elicit minimal detectable action upon the renal vessels, frequently causes the glomerular tuft to enlarge and to appear more densely congested with blood cells. This action may result from constriction of the efferent vessel, either at the point of reassembly of glomerular capillaries or further along its course.

In slightly larger dosage, adrenalin decreases the number of active glomeruli and the number of visibly patent pathways in single glomeruli. These effects are due to action on the arterioles.

8. These observations provide evidence upon which to re-introduce into considerations of renal physiology the conception held by Hermann and doubtless by Ludwig that the extent of filtration surface in the kidney is variable and a factor which must be of major importance in the adjustment of renal function to excretory requirement.

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OBSERVATIONS ON THE COMPOSITION OF GLOMERULAR URINE, WITH PARTICULAR REFERENCE TO THE PROBLEM OF REABSORPTION IN THE RENAL TUBULES[1]

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The conception that a process of absorption occurs in the tubules of the kidney originated in the mind of Carl Ludwig (1) as a consequence of his belief that the fluid separated from the blood in the Malpighian body is a protein-free filtrate from plasma. Such a process only could explain the differences in composition of normal urine as compared with a filtrate from normal blood. His earliest idea was that the force concerned in the process was osmotic pressure, but after it had been shown

[1] This paper without essential alterations was awarded the Boylston prize of the Harvard Medical School in March, 1923. The Board of Award "does not consider itself as approving the doctrines contained in any of the dissertations to which premiums may be adjudged." The results have been presented and the technique demonstrated before the Physiological Society of Philadelphia on December 19, 1921, before the American Physiological Society on December 29, 1921 (Wearn: This Journal, 1922, lix, 490), before the Thirteenth International Congress of Physiology at Edinburgh on July 24, 1923 (Wearn, Schmidt and Richards: Quart. Journ. Exper. Physiol., Suppl. Volume, December, 1923, p. 235; Wearn and Richards: ibid., p. 236) and to numerous visitors to this laboratory.

Acknowledgment should be made to Prof. Robert Chambers of Cornell University, whose demonstration of his capillary pipette before the American Association of Anatomists at their Philadelphia meeting in April, 1921, aroused the thought that similar technique might be applicable to a variety of problems concerned with the frog's kidney. Doctor Wearn is responsible for the suggestion that it might be possible to collect fluid from Bowman's capsule by its aid.

In certain respects our observations concerning the behavior of indigo carmine and phenolsulphonephthalein duplicate those recently published by Bieter and Hirschfelder (THIS JOURNAL, 1924, lxviii, 326). Professor Hirschfelder visited this laboratory during the summer of 1921 and was shown the frog's kidney preparation as it had been developed by Richards and Schmidt up to that time. We had no intimation of his work until after the publication of his reports before the Society of Experimental Medicine and Biology, April 22 and May 10, 1922. Our experiments were begun in October, 1921, and their progress was reported by Wearn (vide supra) before the American Physiological Society on December 29 of that year. In advancing the claim for priority of publication of the first "absolutely objective evidence" in support of tubular reabsorption, Professor Hirschfelder is apparently unaware of Wearn's report.

that the osmotic pressure of urine is higher than that of blood (2) he modified his view to include differential reabsorption of water and solids through the epithelium of the tubules (3).

The earlier work on the physiology of the kidney in the Ludwig laboratory was directed more particularly at the nature of the glomerular function than of tubular. In a review of this work it seems implicit that if filtration is proved to be the characteristic feature of glomerular function, tubular reabsorption must be accepted as a necessary consequence. This justifiable implication is apparent in much of the more recent literature.

The most important objections which have ever been raised against the theory of reabsorption in the tubules are those which were advanced by Heidenhain. In experiments designed to trace the passage of the dye, indigo-carmine, from blood into urine he observed the deposition of dye in the lumen of the tubule, the presence of colored granules in the epithelial cells of the tubule and no trace of the dye in the glomerular structures (4). This fact, together with similar observations on urates, impelled him to the view that the function of the tubules was secretory. The fact that no dye was present in the glomerular tissues or in the capsule of Bowman was one of the reasons which led him to deny the validity of the filtration doctrine and to ascribe secretory power to glomerular as well as to tubular epithelium. In his classical review of the subject of renal function in Hermann's Handbuch he showed how inadequate, on the basis of available data, the filtration theory was to explain the formation of normal urine (5). His figures have since been widely quoted.

Assuming a daily urinary output of 35 grams of urea in 2000 cc. of urine, derived from blood containing 0.025 per cent of urea and capable of yielding a glomerular filtrate containing 0.05 per cent, it is clear that the separation of 70 liters of filtrate would be necessary to rid the blood of 35 grams of urea. Of this, 68 liters must be reabsorbed. Blood flow through the kidney, calculated on the assumption that the kidney receives blood in the proportion which its weight bears to the rest of the body, amounts to 130 liters daily. Clearly, if these assumptions are sound, the separation of 70 liters of filtrate is incredible.

Cushny (6) has exposed the fallacies of this argument. It is cited because of the weighty influence which it has exerted against the acceptance of belief in reabsorption as a predominating influence in the elaboration of urine.

As has already been stated, evidence for glomerular filtration may be regarded as indirect evidence for tubular reabsorption. It is beyond the scope of this paper to outline the mass of evidence accumulated since Heidenhain's time, which permits many physiologists to accept the filtration hypothesis and which has resulted in the development of the

"modern theory" of urine formation (7) which accepts filtration as a fundamental postulate. It is proper, however, to examine briefly the most outstanding experimental evidence which bears directly on the view that reabsorption occurs in the tubules.

1. Among the first and certainly most direct experiments directed at the question of reabsorption in the tubules were those of Ribbert (8). He excised as completely as possible the medulla of one kidney of the rabbit after having removed the other. Recovery was permitted and the urine eliminated was compared with that from control animals which had been subjected to identical operative procedures with the exception of medullary extirpation. The animals survived the operation for forty-eight to sixty hours: blood disappeared from the urine in from four to five hours. Albumin test showed opalescence only. The volume of urine was from two to three times as great as that of the control animals.

These experiments were unsuccessfully repeated by Boyd (9), but later were successfully repeated by Hausmann (10), working under Meyer's direction. Hausmann's results were comparable with those of Ribbert.

The conclusion, drawn from these experiments, was that a dilute fluid is eliminated in the cortex, which in the normal kidney is concentrated by reabsorption of water.

2. von Sobieranski (11) in 1895 repeated Heidenhain's experiments on the elimination of indigo-carmine by the tubules. When Heidenhain's technique was rigidly followed results identical with his were obtained. If, however, larger amounts of dye were introduced or if the animal's tissues were depleted of water before the experiment, the glomeruli were stained and dye was present in Bowman's capsule.

He then discovered that sections of fresh kidney tissue immersed in the serum taken from animals which had been injected with the dye stain very slowly. Hence it seemed clear that the cells of the kidney which are stained by the dye during life must be subjected to stronger concentrations of the dye than those existing in plasma. If the dye is eliminated in a glomerular filtrate which becomes concentrated in the tubule by reabsorption of water, a satisfactory explanation of the discrepancy mentioned would be obtained. Study of the behavior of another dye, sodium carminate, confirmed this thought, and supported the view developed by von Sobieranski that the coloration of tubule cells observed by Heidenhain was caused by contact with the concentrated solution in the lumen of the tubules rather than by the dilute solution in the blood.

A study of the action of diuretics upon the distribution of the dye in the kidney was made. von Schröder had expressed the belief that caffeine stimulates the secreting epithelium of the tubules. If this were true and if indigo-carmine is secreted into the urine by the tubules then caffeine might be expected to cause intensification of the coloration of

tubule cells by injected dye. The result of experiment shows that under the influence of caffeine, staining of tubules by indigo-carmine is less intense. Upon these observations was based the view that indigo-carmine and sodium carminate are eliminated in dilute solution as a filtrate in the glomerulus which is concentrated in the tubules by reabsorption of water. The staining of tubules is due to contact with the concentrated solution of dye in the tubules: caffeine lessens or abolishes the function of reabsorption.

3. Cushny's experiments (12) on the elimination of sodium sulphate constitute the most influential evidence yet brought forward in support of the reabsorption theory. In them he compared the excretion of sodium chloride and sodium sulphate following the intravenous injection of a solution containing the two salts in isosmotic concentration. During the initial period of maximum diuresis which followed chloride was eliminated in greater quantity than sulphate; during waning diuresis sulphate elimination was greater and persisted longer than that of chloride.

When the rate of urine flow from one kidney was decreased by increasing the ureteral pressure—this was done solely to retard the passage of the glomerular fluid through the tubules—this difference in course of elimination of chloride and sulphate was intensified. A similar result was obtained when urine flow was retarded by partial constriction of the renal artery (13). The interpretation of these results is that while the two salts are eliminated with equal readiness in the glomeruli, the sulphate is reabsorbed with difficulty, if at all, in passage through the tubule; no such obstruction to reabsorption is encountered by the chloride. Injected phosphates and urea show behavior similar to that of sulphates and upon the basis of this behavior is erected the conception of "no-threshold substances" in the "modern theory." It is interesting to note that the urine from the obstructed ureter was more acid than that from the unobstructed, a fact which is important in relation to the much debated question whether acidity of urine arises from secretion of acid or reabsorption of alkali.

4. In another group of experiments comparative chemical study has been made of the cortex and of the medulla of the kidney. Hirokawa (14) determined the osmotic pressure of the cortex of the rabbit's kidney under a variety of conditions and obtained remarkably constant values. Similar estimations on the medulla of the kidney gave results which varied with the concentration of the urine which was being eliminated. Hirokawa's figures point to the elimination of a fluid of relatively constant composition in the cortex which is modified in its passage through the medulla. Since the medullary loops of the renal tubules have never been regarded by the advocates of the secretion theory as the chief seat of the secretory process, the conclusion drawn from these experiments is that the nature of the process in the medulla is reabsorptive.

Grünwald (15) fed rabbits with a diet poor in chlorides until the urine became chloride-free. The administration of diuretin to such animals produced a reappearance of chloride in the urine in considerable quantities. In a number of instances death followed the administration of diuretin: appropriate experiments showed that this was due to loss of chlorides. In acute experiments it was found that any renal vaso-dilator was capable of producing a similar result. As in Hirokawa's experiments it was found that the salt content of the cortex was relatively constant; that of the medulla variable. These experiments are regarded by their author as support to the theory of reabsorption.

An observation, analogous to those of Grünwald, was made by Richards and Plant (16), viz., that the production of a renal plethora, by ligation of coeliac axis and the superior and the inferior mesenteric arteries, in a rabbit whose diet had been such as to yield chloride-free urine, caused immediate diuresis and chloride elimination.

In Nishi's experiments (17) the sugar content of the cortex was compared with that of the medulla. Concentration in the cortex varied from 0.01 to 0.066 per cent. If hyperglycemia without glycosuria was produced it varied from 0.04 to 0.06. In neither instance did the medulla show any sugar. If glycosuria was produced, both cortex and medulla contained sugar, the latter, however, in smaller amounts. These results certainly are indicative of a reabsorption of sugar in the medullary portion of the kidney.

5. Two series of experiments made by Bainbridge and his collaborators (18) were designed to bear directly upon this problem. Arrangements were made for perfusing the frog's kidney with oxygenated Ringer's solution through the renal arteries, through the renal-portal vein or through both. The concentration of the fluid issuing from the ureter was measured by the refractometer. Perfusion through the renal arteries resulted in the excretion of fluid through the ureter which was hypotonic to the perfusing fluid. This result was not modified by the additional introduction of perfusion through the renal-portal vein. When the tubules had been poisoned by the introduction of mercuric chloride in very dilute solution via the renal-portal vein the fluid issuing from the ureter was isotonic with the perfusing fluid. In a few instances the dead kidney or the kidney poisoned with mercuric chloride perfused under identical conditions also yielded hypotonic ureteral fluid.

The deductions drawn from these experiments are that isotonic fluid is separated in the glomeruli of the perfused frog's kidney and that this fluid is rendered more dilute during passage through the tubules by the reabsorption of salt. It may be added that in these experiments no evidence was obtained that the cells of the tubule possessed the power of forming urine or of adding anything to the fluid eliminated by the glomeruli.

Hamburger and Brinkman (19) showed that under certain circumstances the perfused frog's kidney forms urine containing no glucose from a perfusing fluid containing as much as 0.08 per cent. Broemser and Hahn (20) obtained similar results (0.07 per cent). These authors interpret their results as evidence that the glomerulus is impermeable to glucose below the concentrations stated; not that it is reabsorbed from the tubule.

In Clark's experiments (21) the renal arteries and renal-portal vein of the frog's kidney were perfused according to the technique of Bainbridge, Collins and Menzies with Hamburger's modification of Ringer's solution. When the perfusing solutions contained glucose in concentration of 0.052 per cent or less, no glucose appeared in the urine: when the concentration was greater than 0.052 per cent and less than 0.23 per cent the glucose content of the urine was lower than that of the perfusion fluid by 0.05 to 0.06 per cent. When the renal-portal perfusion contained 0.4 to 0.5 per cent glucose and the arterial less than 0.1 per cent (the tubules, therefore, being bathed with an intermediate concentration) the glucose content of the urine was less than that of the arterial fluid as long as the content of the mixture issuing from the vein was less than 0.21 per cent: when it was higher than this, the concentration of urinary glucose was exactly the same as that of the arterial fluid. Having confirmed the finding of Atkinson, Clark and Menzies (22) that glucose is not eliminated by the tubule, Clark concluded that glucose passes freely through the glomerular membrane and is reabsorbed from the tubule to a degree which is dependent upon the glucose content of the fluid bathing it.

Bieter and Hirschfelder (23) have published the results of experiments in which the passage of indigo-carmine and phenol-sulphonephthalein through the glomerular membranes of the frog's kidney has been observed directly by the method of Richards and Schmidt. The subsequent accumulation of highly colored fluid in the tubules together with the fact that no such accumulation occurs in the tubules in parts of the kidney in which the glomerular circulation (but not the tubular) has been abolished gave direct evidence of reabsorption of water in the tubule. Despite the fact that their final publication has anticipated ours, we think that, without injustice to them, we may properly regard their results as confirmatory of ours (see footnote p. 209).

The experiments which have been cited constitute the most direct evidence available before the completion of our work upon which to base belief that a process of reabsorption occurs in the renal tubules. Its weight becomes more impressive when it is considered in conjunction with that which has been obtained in favor of the filtration theory of glomerular function. In itself each experiment or group of experiments

is convincing when viewed in the light of the author's interpretation. When subjected to criticism, however, it is discovered that few of the cited experiments are so free from ambiguity as to compel belief in the deductions drawn from it. Thus the experiments of Ribbert are vitiated by the fact that operative reduction in mass of the kidney is followed by diuresis irrespective of medullary extirpation (24). Acceptance of Cushny's views depends on belief that the glomerular fluid is a filtrate and contains sulphate and chloride in the same concentrations as those which obtain in the blood plasma: one is puzzled to know the bearing of the fact that sodium sulphate, in contrast with sodium chloride, was one of the substances found by Barcroft and Straub (25) to increase metabolism in the kidney. von Sobieranski's results were obtained with a dye, foreign to the organism, and are rendered ambiguous by the difficulty of deciding whether intra-vital staining of renal cells really represents passage of dye through the cell and if so in which direction.

The Nishi experiments are clouded by failure to estimate residual blood in cortex and medulla; it is possible that differences in sugar content might be related to differences in blood content. Bainbridge perfused the frog's kidney with Ringer's solution, a fluid which cannot be regarded as physiologically equivalent to blood. In some instances dead kidneys seemed to possess the same power as living. The refractometric method of estimating concentrations presents opportunity for error. Clark's experiments are beyond criticism and those of Bieter and Hirschfelder seem conclusive: they were not published until after the majority of our experiments were completed.

The alternation of authoritative opinion concerning the nature of renal functions, the masses of inconclusive evidence and the predominance of hypothesis over fact in the literature bear testimony to the difficulty of designing experiments upon the kidney of which the result shall be unambiguous and crucial. Intricacy of structure and compactness of mass have prevented the development of analytical experiments of compelling force.

In the work now to be described it is hoped that experiments of unequivocal meaning in a limited field of renal physiology are supplied. They bear directly upon the question of reabsorption of sugar and of chloride in the tubules and upon the place of elimination of certain dyes which have been important reagents in past study of renal physiology; and indirectly upon the question of glomerular filtration. They consist of comparative observations upon the composition of fluids simultaneously taken from Bowman's capsule and from the urinary bladder of the living frog.

METHODS. Healthy frogs, usually Rana pipiens, were used in all the experiments. The brain was pithed with precautions to prevent hemor-

rhage and a long incision made in the body cavity from the pelvic region upward, usually through the shoulder girdle. The anterior abdominal vein was ligated and cut, and in experiments in which intravenous injection was required a cannula was inserted pointing toward the heart. The animal was fastened to a board of convenient size, through which a small metal tube (8 mm. diameter) passed so that its upper end projected a few millimeters above the surface of the board. When female frogs were used, the ovaries were excised, hemorrhage being prevented by ligature or cautery of vessels. The cauterized skin and muscles of the abdominal wall on the right side were divided by transverse incision at the level of the kidney extending around to the vertebral column. The peritoneum was divided in a line running parallel to the lateral border of the right kidney. By means of small clips attached to the cut edge of the renal peritoneum, the lateral border of the kidney was drawn so that a portion of it lay over the end of the tube passing through the board. The board was placed upon the stage of a binocular microscope. A small arc lamp was so placed that its rays, after passing through a jar of cold water tinged with methylene blue, were focussed on the mirror and then reflected upward through the tube upon which the lateral border of a portion of the kidney lay. By such procedure the vascular structure of the kidney becomes clearly visible. This method is a modification of that devised by Richards and Schmidt (26) for observation of the glomerular circulation.

The essential part of the apparatus used for drawing fluid from Bowman's capsule is a sharp pointed capillary pipette. A small piece of quartz tubing, 1 mm. in diameter, was drawn out in a flame and broken at the narrow part.[2] The point (10 to 20μ inner diameter) was either sharpened on a stone or broken in such a way that a sharp edge or spicule projected from the tip. This quartz pipette was sealed into the end of one arm of a 3-way glass stop-cock tube by means of wax. A piece of thick walled rubber tubing, 3 to 4 inches long, closed at one end by a bit of glass rod was attached to the opposite arm. The third arm, projecting at right angles to the other two, was connected with a glass levelling bulb by 2½ feet of rubber tubing. By means of the levelling bulb the whole apparatus including the quartz tip was filled with mercury.[3]

The pipette was firmly held in a stage of Barbour's pattern capable of giving micrometric adjustment in three planes. The stage in turn was firmly clamped to a Zimmerman stativ, well adapted to coarse adjustments. Figure 1 is a photograph of the apparatus.

[2] Quartz was chosen after it was found that the pressure required to force a glass tip through the capsule of Bowman usually broke the pipette.

[3] It is extremely difficult to manage fluid in a capillary tube if the column of fluid is broken by a bubble of air: hence the necessity of completely filling the pipette with a fluid before insertion.

Insertion of the pipette into the glomerular space was carried out with the aid of the binocular microscope in the following manner. The point of the pipette was brought into the microscopic field directly over the

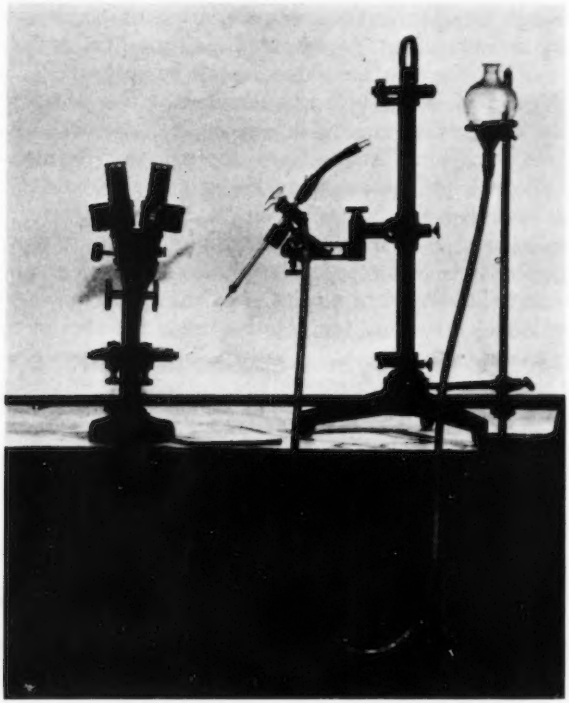

Fig. 1. Photograph of capillary pipette ready for use

Fig. 2. Photomicrographs of a single glomerulus in the living kidney before (A) and immediately after (B) insertion of capillary pipette through its capsule (×71)

capsule to be punctured, so that it was poised about one millimeter above the surface of the kidney. A very fine stream of air, issuing from a small hypodermic needle attached to the microscope stand, was allowed

to play upon the surface of the kidney until the thin layer of fluid on the surface was removed and the field of operation dried. This procedure made it possible to introduce the pipette through the capsule of Bowman without contamination.[4] This drying of the surface helped to overcome to some extent the difficulty encountered in puncturing the capsule because of its elasticity and toughness: it prevented accidental trauma of the capillary tuft or of the inner surface of the capsule opposite the point of puncture. The point of the pipette was directed at the clear space visible between the capillary tuft and the capsule of Bowman. The point of puncture was so chosen that the tip of the pipette after insertion could not exert pressure by contact upon the glomerular vessels. Figure 2 shows a glomerulus before and after insertion of the pipette.

After insertion of the pipette the three-way stop-cock was turned so that the connection between the levelling bulb and the pipette was opened. The bulb was then lowered to a point two or three centimeters below the level of the kidney, thus creating negative pressure sufficient to draw the glomerular fluid into the pipette. To discontinue collection of the glomerular urine the three-way cock was turned so that connection between the collecting tip and the rest of the system was closed. Contamination during withdrawal of the pipette was avoided by again drying the surface of the kidney. The tip of the pipette was carefully rinsed with distilled water immediately after its withdrawal.

Transfer of fluid from the pipette to tubes adapted to chemical testing was carried out as follows: A long glass capillary tube with an inner diameter from three to six times that of the quartz pipette was mounted obliquely upon the microscope stage so that its upper opening was within the field of vision. Using the Barbour stage the tip of the pipette was inserted into the opening and the contents expelled into it by pressure.

After transfer of the glomerular fluid to the glass capillary, division into portions sufficient for single tests was made by drawing it further into the capillary and cutting the tube at the midpoint of the column of fluid. This process was repeated as many times as the volume of the original sample justified.

To add a reagent to any portion of the glomerular urine it was sufficient to touch the end of the capillary to the surface of the reagent and thus permit capillarity to force it into the tube. Mixture of urine with reagent was accomplished by moving the fluids back and forth in the tubes by gentle suction and pressure. In tests which required heat, the ends of the capillary were sealed in a flame, and the tube immersed in boiling water.

[4] For the suggestion which led to the development of this method of avoiding contamination we are indebted to the friendly interest of Dr. William Pepper.

During the collection of glomerular fluid, urine accumulated in the bladder which had been emptied at the beginning of the experiment. This bladder urine was taken at the end of the experiment and subjected to tests identical in method with those applied to the glomerular urine.

RESULTS. *Protein.* The acetic acid-potassium ferrocyanide test was used. Samples of glomerular fluid and of bladder urine from eleven different frogs were tested and in none was a precipitate formed. This result is important not only from the standpoint of the physiology of the kidney, but also because it indicates that the methods used to avoid contamination with tissue fluid were successful. As a control, a small portion of the fluid which normally covers the surface of the kidney was collected, diluted one hundred times with water, and subjected to test in a capillary tube. An easily distinguishable, flocculent precipitate was obtained. Frog's plasma, diluted 1:100, yielded a similar result. These control tests indicate the delicacy of the method employed and proved that there was neither significant contamination of the pipette during its insertion or withdrawal and that there was no leakage of tissue fluid into the capsule of Bowman during collection.

The eleven samples of glomerular fluid referred to were taken from glomeruli through whose vessels blood flow was rapid. In a number of other instances collection was attempted from glomeruli through whose capillaries circulation was sluggish. In these the fluid accumulated slowly in the pipette, but no test upon it was possible, for when attempts were made to transfer it to the glass capillary tube it was found to be clotted. In view of the repeated occurrence of this phenomenon it seemed probable that under these circumstances a leakage of plasma through the capillaries had occurred.

Sugar. Benedict's qualitative solution was introduced into the capillary tubes in volume five to ten times that of the sample of glomerular or bladder urine to be tested. The ends of the tube were sealed in a flame and the tube immersed in boiling water for five minutes. Control tests showed no precipitate or discoloration of the reagent with distilled water; glucose solutions of 0.02 per cent and upward on the other hand showed unmistakable reduction. In each experiment, the reduction tests on glomerular and bladder urines were made simultaneously and under the same conditions. In every case, a control test of the reagent with distilled water was made at the same time. In many instances several tests on the bladder urine were made in which the proportion of reagent to urine varied from 1:1 to 10:1.

In the first two experiments no reaction could be obtained with either glomerular or bladder urine. Sugar estimations were then made on the blood of nine frogs by McLean's method (27) with the result that no

sugar was detectable. (These experiments were made in November and December.)

Therefore, in subsequent experiments, with the exception of nos. 15 and 16 (made in March), glucose was injected in varying amounts under the skin of the thigh before beginning the collection of glomerular and bladder urine. The details of the majority of experiments are contained in Table 1. The results of seven experiments in which blood sugar was estimated are grouped below:

NUMBER	BLOOD SUGAR	SUGAR IN	
		Glomerular urine	Bladder urine
	per cent		
10	0.0175	+	0
5	0.022	+	0
15	0.025	+	0
6	0.033	+	0
16	0.037	+	0
7	0.05	+	0
4	0.065	++	+

In all in which blood sugar was 0.05 per cent or lower, glomerular urine reduced Benedict's solution; bladder urine did not. In the single experiment in which blood sugar was found to be higher than 0.05 per cent both fluids reduced Benedict's solution, glomerular urine more intensely than bladder urine.

Two experiments (8 and 9) made in December are included in table 1 in which no glucose was injected and no reduction was produced by either glomerular or bladder urine.

In the experiments 4, 12 and 14 positive reduction tests were obtained with both the glomerular and bladder urines.

The number of results at present available is insufficient to permit a statement of the exact threshold level of sugar.

Figure 3 shows a photomicrograph of tubes in which glomerular fluid and bladder urine have been subjected to the test for sugar as described.

Chlorides. The test used was silver nitrate and nitric acid. When performed in capillary tubes, it produced visible turbidity with NaCl concentrations as low as 0.01 per cent. It has been shown by van der Heyde (28) that the urine of fasting frogs is nearly chloride-free. Urine collected from several frogs from the stock used in these experiments yielded only turbidity or opalescence when tested grossly with silver nitrate. In the first comparison of glomerular fluid with bladder urine, the former yielded a heavy white precipitate, the latter a very small amount.

TABLE 1

NUMBER OF EXPERIMENT	WEIGHT AND PREPARATION OF FROG	TIME OF COLLECTION OF GLOMERULAR URINE	GLOMERULAR URINE			BLADDER URINE			BLOOD	
			Protein	Sugar	Cl	Protein	Sugar	Cl	Sugar	NaCl
		hours							*per cent*	*per cent*
1	55 grams. 2 cc. 5 per cent glucose in stomach during operation		0							
2	2 cc. 1 per cent glucose + 3 cc. H_2O subcutaneously immediately before operation	3	0			0				
3	Same frog as in experiment 2. Another capsule punctured	3		+			0			
4	1.3 cc. 1 per cent glucose + 3 cc. H_2O subcutaneously immediately before collection began	2		++			+		0.065	
5	0.4 cc. 1 per cent glucose + 3 cc. H_2O subcutaneously 15 minutes before operation.	$3\frac{1}{4}$		++					0.022	
	Another capsule punctured	$\frac{1}{2}$		+			0			
6	Small frog. 1.5 cc. 1 per cent glucose 12 hours before operation. 4 cc. H_2O subcutaneously after pithing	2	0	+	+	0			0.033	
7	45 grams. 0.3 cc. 1 per cent glucose 5 hours before operation	4	0	++	+	0	0	0	0.05	
8	48 grams. No injection		0	0	+	0	0	0		0.21
9	35 grams. 4 cc. 0.05 per cent NaCl subcutaneously immediately before operation	$4\frac{3}{4}$	0	+	++	0	0	+		0.215
10	48 grams. 0.3 cc. 1 per cent glucose + 3 cc. H_2O 15 minutes before collection began	10*	0	+	++	0	0	±	0.0175	
11	In distilled water 20 hours before experiment 0.3 cc. 1 per cent glucose immediately before operation	5	0	++	++	0	0	0	0.045	
12	48 grams. 0.6 cc. 1 per cent glucose + 4 cc. H_2O immediately before collection began		0	++	++	0	+	0		
13	54 grams. 0.4 cc. 1 per cent glucose + 3 cc. H_2O subcutaneously immediately before operation	3		+	++		0	0		
14	32 grams. 0.4 cc. 1 per cent glucose + 2 cc. H_2O. Excessive diuresis and very rapid collection of glomerular urine	4†		++	+		+	0		
15	2 cc. 0.4 per cent NaCl subcutaneously before operation	3		+			0		0.025	
16	No injection	2		+			0		0.037	

* 2.4 mgm.

† 10 to 12 mgm.

When the frogs were kept in distilled water for 24 hours before collection, bladder urine gave no reaction for chloride. Experiments 7 to 13 in table 1 show the results of comparative tests of glomerular urine and bladder urine. In five, no reactions were obtained in the latter; strong reactions in the former. Figure 4 is a photomicrograph showing chloride tests on glomerular and bladder urine from such a frog.

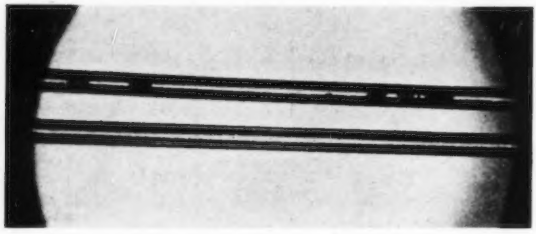

Fig. 3. Photomicrographs showing Benedict's test for sugar in glomerular urine (upper tube) and bladder urine (lower tube).

Urea. A number of comparative tests for urea were made upon the glomerular urine and bladder urine, using the xanthydrol[5] reagent prepared by the method described by Oliver (29). Typical crystals, similar to those pictured by Oliver were formed in both fluids, much more abundantly however in the bladder urine. We accepted this as evidence that

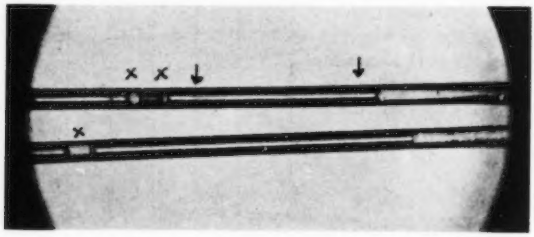

Fig. 4. Photomicrograph showing tests for chloride in glomerular urine (upper tube) and bladder urine (lower tube). The columns of fluid are broken by air bubbles at the points marked *x*. In the upper tube, the fluid between the air bubbles was packed with a precipitate. A heavy turbidity was visible between the points marked ↓.

urea is eliminated through the glomerular membranes, but discontinued the tests because of the feeling that effort should be devoted to development of methods which should yield results more reliable and more nearly quantitative when applied to these minute amounts of fluid.[5]

[5] We are indebted to Dr. J. B. Brown for help in making the xanthydrol and the cobalt sodium nitrite reagents as well as for other chemical assistance.

Potassium. Using the cobalt-sodium nitrite reagent of A. B. Macallum (30), potassium was detected in both glomerular and bladder urines. When, however, the frogs were removed from the tanks through which tap water flowed and kept in distilled water, frequently changed, for three days before an experiment, no potassium was found in either glomerular or bladder urine.

Reaction. In a number of experiments attempts have been made to test the reaction of the glomerular fluid in comparison with that of blood plasma and of bladder urine. In one experiment a frog, weight 62 grams, was injected subcutaneously before the operation with 2 cc. of 3 per cent sodium bicarbonate solution. Glomerular and bladder urines were then collected for eight hours: at the end of this time another capsule was punctured and collection continued for ten hours more. The fluids were tested in capillary tubes made of quartz, cleaned with the most extreme care. A portion of each was mixed with a dilute solution of neutral red in the proportion of 1:3. Both samples of glomerular urine caused change of color to yellow with the deposition of small yellow crystals. A phosphate solution having a known pH of 7.5, similarly tested, gave a similar result. Both samples of bladder urine mixed with the reagent were pink and no crystals were deposited. A phosphate solution of pH = 6.6, similarly tested, behaved similarly. These experiments were discontinued for the same reason as that stated in connection with urea.

In experiments made by one of us since the appearance of Bieter and Hirschfelder's paper it has been observed that when either phenolsulphonephthalein or neutral red is injected into the circulation of the living frog, the color of the fluid which is to be seen in the capsular space corresponds to a reaction distinctly more alkaline than that represented by the color of the dye as it is to be seen in the tubules.

Dyes. Indigo-carmine is of special interest in the study of the kidney because it is the dye which was chosen by Heidenhain for tracing the path of elimination from blood into urine. In sections of the kidney he found it in the tubules and not in the glomerulus; hence he revived the Bowman theory of tubular secretion and denied the filtration hypothesis. The force of Heidenhain's observations and arguments has diminished with the accumulation of further evidence (27).

In the study here presented opportunity was afforded for directly observing in the living tissues concerned the events which follow the injection of this dye into the blood stream.

Arrangements were perfected for injection of the dye into the anterior abdominal vein while an observer was looking at the kidney. At the same time the capillary pipette was projecting into the capsule of Bowman so that both glomerular fluid and bladder urine were collected during the elimination of the dyes.

The sequence of changes which was seen to follow the injection of 0.5 cc. of a 0.5 per cent indigo-carmine solution (Grübler) in water or salt solution is as follows: Within the first thirty seconds after injection the visible arteries become blue: immediately the capillary tuft in the glomerulus becomes intensely blue: then the colorless, transparent, fluid-filled space between tuft and capsule takes on a light blue tinge: then all of the visible vessels, including veins, take on the color so that the whole kidney is opaque: finally the color fades rapidly so that the original state of the kidney is restored. This sequence is complete in 3 to 5 minutes following the injection.

Within 10 to 30 minutes later another change is seen. The tubules which are seen with difficulty in the unstained kidney on account of their transparency become more clearly visible owing to the collection of blue granules in their walls. Presently thread-like collections of dye can be discovered apparently within the lumina of some tubules; what seems to be a stippling of the inner border appears in others.

The glomerular fluid which was collected during the series of events described was blue: so also was the bladder urine. When approximately equal amounts of the two fluids were discharged upon white filter paper, the stain produced by the glomerular urine was distinctly blue, but was less intense than that produced by the bladder urine.

While the complete interpretation of these changes cannot be made out with certainty at the present time, one fact is clear: indigo-carmine is eliminated by the glomerulus.

The complete temporary disappearance of the dye points to the important influence which the fact of its reduction to a leuco-base may have exerted in the past in the development of diversity of opinion regarding the place of its elimination. The character of its distribution in the tubules as has been described is in harmony with von Sobieranski's belief that the staining of tubule cells occurred as a result of the presence of the dye in the lumen of the tubule in more concentrated solution than that in which it exists in blood.

Phenolsulphonephthalein (phenol red) is the dye which was introduced by Rowntree and Geraghty (32) as a clinical test of renal function. Their studies on frogs led them to the belief that it was eliminated by the tubules and not by the glomeruli: and in the voluminous clinical literature it is commonly alluded to as a test of renal "secretion." In one experiment in which phenol red was injected intravenously (0.5 cc. of 0.5 per cent solution) and in another in which the injection was subcutaneous, glomerular fluid subsequently collected became distinctly red when treated with dilute alkali. The intensity of the stains made by discharging approximately equal amounts of glomerular urine and bladder urine, simultaneously collected, upon alkaline filter paper was approximately the same.

Methylene blue in similar experiments was identified in glomerular urine.

Other observations. Choice of a glomerulus for puncture and collection of urine was determined on the basis of its accessibility and the rate of blood flow through its vessels. It was assumed that the more rapid the blood flow the greater would be the rate of glomerular elimination. At the instant when the pipette pierced the capsule of Bowman, and particularly if a capillary loop was touched by the tip of the pipette, flow of blood through that glomerular tuft ceased for an interval of from a few seconds to two minutes. During the intermission of blood flow fluid did not rise in the pipette, a fact which not only indicates that formation of glomerular urine ceases with glomerular blood flow but also that the fluid drawn from the glomerulus in these experiments did not originate in the tubule.

The length of time during which collection of fluid could be continued from a single glomerulus was surprising. In one preparation the experiment lasted for eighteen hours. In this instance the total fluid collected amounted to 10 milligrams. On another occasion more than 6 milligrams of urine were collected, following injection of glucose, in four hours. These figures are not as great as those calculated by Cushny to represent the performance of a cat's glomerulus, but the difference is no greater than one might anticipate in view of differences in glomerular capillary pressure in the cat and frog. It is, of course, not to be assumed that all of the fluid eliminated from the blood is collected in the pipette.

Discussion. Extended discussion of the results here presented is unnecessary. Direct testing of the fluid eliminated by the frog's glomerulus proves the assumption which was made by the earliest of the modern students of renal physiology, that a protein-free, watery fluid is separated from the blood stream as it passes through the glomerular capillaries. Absence of protein from the glomerular fluid constitutes serious objection to de Haan's hypothesis of the normal permeability of the glomerular membranes to colloids (33).

The discovery that two substances, sodium chloride and glucose, both of which are normal constituents of blood plasma, are not to be found in bladder urine under the conditions of these experiments, but are to be found in considerable concentration in the fluid taken directly from the glomerulus, proves beyond doubt that reabsorption of these substances must take place in the renal tubules. Proof is also at hand in these experiments that the threshold of reabsorption of these two substances is not the same. The conception of differential reabsorption in the tubules therefore receives support of the most direct character.

Clark's criticisms of Hamburger's views on the partial permeability of the glomerular membrane to glucose are supported by our work. Ham-

burger dismissed the possibility of reabsorption of glucose from the tubules and sought to explain the renal retention of glucose by glomerular impermeability. His conclusions on this point and on the behavior of other sugars in the kidney seem to require radical revision.

The observations yield evidence that two dyes, study of which has had important influence in the development of the secretory theory of urine formation, are certainly eliminated in the frog's kidney by way of the glomerulus. They therefore add materially to the evidence advanced by others which indicates that it is not necessary to conceive of a vital secretory process which takes place in the cells of the tubules, as necessary for the elimination of these substances.

The experiments do not contain direct evidence concerning the nature of the process by which the protein-free fluid is separated from the blood in the glomerulus. The fact that sugar and sodium chloride are both there eliminated, only to be subsequently reabsorbed, indicates that the glomerular process is not one which is adjusted to the changing needs of the organism. It indicates that the further elaboration of urine which is accomplished in the tubules is the process or group of processes by which the kidney is so admirably adapted to the performance of its part in the regulation of constancy of composition of the body fluids. If this is true it may be regarded as indirect evidence that the process in the glomerulus is physical and since the most conspicuous physical force demonstrable in the glomerulus is the blood pressure, it may be regarded as evidence in favor of the filtration hypothesis.

It will be objected that the observations here reported were made on the frog's kidney which is a mesonephros, and hence is different in the details of its structure from that of mammals; that there is no guarantee that these observations are applicable to events in the mammalian kidney. That there is a striking similarity of structure between the glomerulus of the frog and that of mammals no one will deny; that the mesonephros of the frog serves that animal in the same fashion and as effectively as the kidney serves the higher mammals is also hardly open to contradiction. Until observations of similar directness have been made upon mammalian kidney, with contrary results, it is believed that the inferences drawn from these experiments may be extended to the mammalian kidney.

SUMMARY

1. A technique has been devised for simultaneous collection of glomerular and bladder urine in frogs.

2. The glomerular fluid is free from protein when blood flow through the glomerular capillaries is rapid. It may contain protein when blood flow is sluggish and the capillaries are dilated.

3. Starving frogs in winter showed absence of sugar from the blood. When a blood sugar concentration less than 0.05 per cent was established by subcutaneous injection of glucose glomerular urine was found to contain sugar while bladder urine did not.

4. Starving frogs kept in distilled water eliminated a bladder urine which was free from chlorides. The glomerular urine of those frogs contained chlorides.

5. Preliminary tests of the urea content and of the reaction of glomerular urine have been made.

6. The dyes, indigo-carmine, phenolsulphonephthalein and methylene blue, are eliminated from the frog's circulation by way of the glomerulus.

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